Consequences of drought, flooding, and insect herbivory on the distributions of closely related willow and poplar (Salicaceae) species across hydrologic gradients in Minnesota wetlands

A Dissertation
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Xiaojing Wei

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Jeannine Cavender-Bares

September 2016

Acknowledgements

I thank my advisor, Jeannine Cavender-Bares for her strong supports and constant encouragements, which has been important for me to overcome various obstacles along my graduate school journey. I am also very grateful to my three other committee members, Elizabeth Borer, Sarah Hobbie, and George Weiblen, who provided helpful advices on my dissertation in various aspects. Don Alstad was a former committee member of mine and was a strong support during my first three years in graduate school.

I was fortunate to have the opportunity to collaborate with several fabulous ecologists in my dissertation. Jessica Savage introduced me to the Salicaceae system and was the leader of the initial set up of the field experiment; her love for the Salicaceae plants was contagious. Rick Lindroth and Ken Keefover-Ring provided protocols and standards of the defense compounds analysis (Chapter 2). Martin Volf helped with the field experiment and the chemistry analysis (Chapter 1& 2). Charlotte Riggs provided soil nitrogen availability data in the field experiment (Chapter 1).

Besides these collaborators, I have also received helps from many other people. Jackson Struble and Andrew Stone were two wonderful research assistants working on the field (Chapter 1 & 2) and greenhouse experiments (Chapter 3). I am also thankful to the staff and interns working at Cedar Creek Ecosystem Science Reserve for their helps on field works (Chapter 1 & 2). Without them, my field seasons would be much harder. The members of the Cavender-Bares Lab have been great colleagues. They not only provided feedbacks to my experiment plans, data analyses, and manuscripts, but also helped transplanting cuttings and sewing cages for the field experiment.

Last but not least, I am grateful to the funding agencies that made the work possible. This dissertation was primarily funded by NSF grants awarded to Jeannine Cavender-Bares. I also received a Bell Museum Fellowship supporting the field experiment (Chapter 1 & 2).

Dedication

This thesis is dedicated to my parents, Yuehong Hou and Wei Wei.

Abstract

Understanding the mechanisms underlying species diversity patterns is a central and long-standing issue in ecology. Beta diversity, the variances in species composition among sites, is an important aspect of species diversity that links local diversity patterns to regional diversity patterns. One of the community assembly processes that known to influence beta diversity is environmental filtering. Besides environmental filtering, biotic interactions can also affect beta diversity, if biotic factors exert differential effects on species performances across environmental gradients.

Contributions of different community assembly processes to beta diversity can be tested with multiple approaches. First, experimental approaches allow direct tests of effects of abiotic and biotic factors on species performances across environmental gradients. Second, functional traits can be used to infer the community assembly processes underlying species diversity patterns, as differences in performance responses between species are caused by differences in relevant traits. Last, closely related species tend to (but not always) share similar ecological attributes; therefore, phylogenetic information may be used to predict functional traits and infer community assembly processes.

This dissertation examined the effect of environmental filtering and insect herbivory on distributions of 14 willow and poplar species across hydrologic gradients in Central Minnesota, combining field and greenhouse experiments, functional traits data, and phylogenetic analyses. At our study site, Cedar Creek Ecosystem Science Reserve, the species showed differential distributions across a water table depth gradient, suggesting

environmental filtering mediated by water stresses likely caused habitat segregation among species. Furthermore, species in the Salicaceae family are known to host a variety of insect herbivores, which makes insect herbivory another possible process influencing beta diversity among the Salicaceae communities.

Chapter 1 tested environmental filtering in the Salicaceae species using a field experimental test. Cuttings of the 14 species were transplanted into 40 common gardens established along water table depth gradients in the field, where competition was minimized and herbivory was controlled. Species fitness response to the hydrologic environment was estimated based on cumulative growth and survival over two years using aster fitness models. Variation in nine drought and flooding tolerance traits were examined; these traits were expected to contribute to performance based on *a priori* understanding of plant function in relation to water availability and stress. Fitness variation of each species in the field experiment was used to model their water table depth optima. These optima predicted 75% of the variation in species observed hydrologic niches, based on peak abundances in naturally assembled communities in the surrounding region. Multiple traits associated with water transport efficiency and with water stress tolerance were correlated with species hydrologic niches, but they did not necessarily covary with each other. As a consequence, species occupying similar hydrologic niches had different combinations of trait values. Moreover, individual traits were less phylogenetically conserved than species hydrologic niches or integrated water stress tolerance as determined by multiple traits. In conclusion, differential fitness among species along hydrologic gradients is the consequence of multiple traits associated with

water transport and water stress tolerance, expressed in different combinations by different species. Varying environmental tolerances, in turn, play a critical role in driving niche segregation among close relatives along hydrologic gradients.

In chapter 2, the effect of insect herbivory on the growth of the Salicaceae species across hydrologic gradients was examined using the same common garden experiment mentioned above. An insect exclusion treatment was performed nested within the gardens, by installing real and sham cages to individual experimental plants and comparing species growth in the different cage treatments. Concentrations of nitrogen, carbon, and two groups of defense compounds, phenolic glycosides and condensed tannins in leaves were measured, and phylogenetic signals in these foliar traits were analyzed. The results showed that insect herbivory reduced plant growth, was different between species, and varied across the water table depth gradient in a hump-shaped manner. However, herbivory did not promote habitat segregation among the species because there was no interaction effect between species and water table depth on either herbivory damages or the cage treatment effect on growth. Furthermore, variations in leaf traits could partially explain the variation in herbivory between species but not variation across hydrologic gradients. Last, closely related species did not share similar defense traits: although secondary metabolite richness was phylogenetically conserved, the concentrations of the defense compounds and nitrogen were not. In conclusion, although insect herbivory did not promote beta diversity among the Salicaceae communities across hydrologic gradients, the dissimilarity in defense chemistry might promote the coexistence of close relatives within local communities through density-dependent effects.

In the last chapter, a greenhouse experiment was performed to examine responses of growth and physiological traits in seven willow (genus *Salix*) species to a six-week long flooding treatment followed by a six-week long recovery period. These seven species were selected to represent the full ranges of mean water table depth and season water table depth fluctuation in the natural habitats of the 14 species. The flooding treatment increased plant growth and carbon assimilation by improving plant water status: plants received the flooding treatment showed higher stomatal conductance and predawn leaf water potential than plants received the control treatment. Furthermore, species distributed in wetter habitats had higher stem growth rate in the flooding treatment; and the species distributed in habitats with greater water table depth fluctuations showed greater variations in growth between the flooding and the recovery period. The results of this experiment suggest differential tolerances to flooding and water table depth fluctuation may contribute to habitat segregation among the species.

Table of Contents

List of Tables

List of Figures

Introduction

Understanding the mechanisms maintaining species diversity is a central and long-standing concern in ecology. Beta diversity, the variance of species composition across sites, can substantially contribute to species diversity at medium to large spatial scales (Condit et al., 2002; Tuomisto et al., 1995). One type of drivers for beta diversity patterns is niche-based process (Legendre et al., 2005). Niche-based theories assume that species have different functional attributes that confer advantages in different environments. Such functional differentiation causes species to show differential fitness responses across environmental gradients, which in turn, causes their distributions to be different from each other (Bazzaz, 1996; Cavender-Bares, Jeannine et al., 2004; Chase & Leibold, 2003; Chesson, 2000; Tilman, 1982).

Among niche-based processes, habitat segregation caused by abiotic factors is considered to be particularly important for distributions of sessile organisms, such as plants (Warmings, 1895; Kenkel et al., 1991; Sherman et al., 1998; Kobe, 1999; Silvertown et al., 1999; Reich et al., 2003). A key abiotic factor for terrestrial plants is soil water availability, as both the deficiency and the excess of soil water can exert stresses on plants. Species turnover along soil water availability gradients are found in plant communities at various geographical locations, which suggests environmental filtering driven by differentiation in water stress tolerances among species is a common mechanism influencing plant beta diversity in terrestrial ecosystems (Araya et al., 2011; Silvertown et al., 2015).

Besides abiotic factors, biotic interactions can also promote beta diversity by differentially influencing species performances across environmental gradients (e.g., Fine et al. 2004, 2006). For instance, the effect of herbivory on the performance of host plants is known can vary along environmental gradients (Louda, 1982; 1983; Kelly & Dyer, 2002; Maron & Crone, 2006), as both resistance and tolerance of plants to herbivory can vary with abiotic factors.

In the recent decade, phylogenetic information has been increasingly incorporated into studies of community ecology (Cavender-Bares, J. et al., 2004; Cavender-Bares et al., 2009; Lind et al., 2015; Webb et al., 2002; Weiblen et al., 2006). It is long recognized that closely related species should have similar traits due to their shared evolutionary history (Darwin, 1859). Therefore, it is possible to predict ecological attributes of species based on phylogenetic relationships between species. Such predictions could be particularly useful when measuring traits is logistically challenging. The extent to which phylogenetic relatedness among species could predict their ecological similarity is a current focus of the field (Cavender-Bares et al., 2009; Narwani et al., 2015).

In this dissertation, I examined the effect of environmental filtering and insect herbivory on beta diversity among communities of closely related willow and poplar (Salicaceae) species across hydrologic gradients in Central Minnesota, using a combination of approaches including experiments, traits data, and phylogenetic information. Previous studies found the Salicaceae species had differential distributions across hydrologic gradient. Furthermore, species' hydrologic niches determined based on

their natural distributions showed a significantly conserved phylogenetic signal (Savage et al., 2009; Savage & Cavender-Bares, 2011; 2012).

Building on the previous studies, in Chapter 1, I tested the effect of environmental filtering on the fitness of 14 Salicaceae species across hydrologic gradients by transplanting cuttings of the species into a series of common gardens along natural hydrologic gradients in the field, where completion was minimized and herbivory was controlled. I also examined correlations between drought and flooding tolerance traits and species' hydrologic niches, as well as the phylogenetic signals in the traits. In Chapter 2, using the same common garden experiment, I examined the effect of insect herbivory on species performance across hydrologic gradients by installing sham and real cages on experimental plants and comparing performances between plants received different treatments. I also examined correlations between herbivory damage and leaf traits associated with herbivory resistance, as well as phylogenetic signals in these traits. In the last chapter, I examined flooding tolerance in seven *Salix* species selected from the 14 species used in previous chapters. Cuttings of the species were exposed to a partial-submerging treatment of six weeks, followed by another six-week recovery period. I measured plant growth, biomass allocation, and multiple physiological and morphological traits to access plant performance in the flooding and control treatment, and to examine the possible mechanisms underlying flooding tolerance in these species.

Chapter 1

An experimental test of fitness variation across a hydrologic gradient predicts willow and poplar species distributions

Summary

Environmental filtering is an important community assembly process influencing species distributions. Contrasting species abundance patterns along environmental gradients are commonly used to provide evidence for environmental filtering. However, the same patterns may result from alternative or concurrent community assembly processes, including dispersal and competition. Experimental tests are an important means to decipher whether species fitness varies with environment, in the absence of dispersal constraints and competition, to draw conclusions about the importance of environmental filtering in community assembly. We performed an experimental test of environmental filtering in 14 closely related willow and poplar species (family Salicaceae) by transplanting cuttings of each species into a series of common gardens (40 total) established along natural hydrologic gradients in the field, where competition and herbivory were controlled or minimized. We measured species fitness response to the hydrologic environment based on cumulative growth and survival over two years using aster fitness models. We also examined variation in nine drought and flooding tolerance traits expected to contribute to performance based on *a priori* understanding of plant function in relation to water availability and stress. We found substantial evidence that environmental filtering along hydrologic gradients played a critical role in determining species distributions. Fitness variation of each species in the field experiment was used to model their water table depth optima. These optima predicted 75% of the variation in species observed hydrologic niches, based on peak abundances in naturally assembled communities in the surrounding region. Multiple traits associated with water transport

efficiency and water stress tolerance were correlated with species hydrologic niches, but they did not necessarily covary with each other. As a consequence, species occupying similar hydrologic niches had different combinations of trait values. Moreover, individual traits were less phylogenetically conserved than species hydrologic niches or integrated water stress tolerance as determined by multiple traits. We conclude that differential fitness among species along hydrologic gradients is the consequence of multiple traits associated with water transport and water stress tolerance, expressed in different combinations by different species. Varying environmental tolerances, in turn, play a critical role in driving niche segregation among close relatives along hydrologic gradients.

Key words

Environmental filtering, field experiment, aster fitness models, functional traits, hydrologic niche segregation, phylogenetic signal, Salicaceae (willow and poplar)

Introduction

Understanding the mechanisms of community assembly is a central concern in community ecology and in the maintenance of biodiversity. Environmental filtering has long been recognized as an important assembly process driving shifts in species composition (e.g., Whittaker 1960) and functional attributes along environmental gradients (Diaz et al. 1998, Cavender-Bares et al. 2004, Cornwell and Ackerly 2009). The term "environmental filtering" has been broadly used to refer to the phenomenon in which not all species that arrive in a given location from the regional pool become members of the local community; rather, only those with appropriate characteristics establish and persist in the habitat, given the environment (Van der Valk 1981, Woodward and Diament 1991, Bazzaz 1991, Weiher and Keddy 1995, reviewed in Kraft et al. 2015a). One challenge in studying environmental filtering is the separation of the effects of multiple community assembly processes on observed species distribution patterns. Abiotic factors and biotic interactions can both act as environmental filters because they can change along environmental gradients and exclude species from unsuitable habitats. For instance, Fine et al. (2004, 2006) found that the damaging effect of herbivory became more severe on species native to nutrient-rich soils when they were transplanted into nutrient-poor soils, restricting these species to their native habitats. Beyond herbivory, interactions among neighboring plants, which significantly affect plant survival and reproduction, have been shown to shift from competition to facilitation along stress gradients (Callaway et al. 2002). Finally, differential dispersal (Ehrlén and Eriksson 2000, Tuomisto et al. 2003) and pollination constraints (Sargent and Ackerly 2008, Chalcoff et al. 2012) have also been shown to drive contrasting distribution patterns among species across environmental gradients.

To differentiate effects of these related but different processes on community assembly, Kraft et al. (2015) recommended that the use of the term "environmental filtering" be restricted to filtering processes caused by abiotic factors (hereafter we will use "environmental filtering" in this narrow sense) and that stringent criteria be used to test for environmental filtering. To date, observations of shifts in species abundances along environmental gradients are the most frequently used evidence for inferring environmental filtering (Kraft et al. 2015). However, such patterns, when used alone, cannot differentiate shifts in species abundance caused by abiotic factors from alternative community assembly processes that vary along environmental gradients.

Distributions of functional traits within and among communities along environmental gradients have been used as corroborating evidence supporting conclusions about environmental filtering (Diaz et al. 1998, Weiher et al. 1998, Cavender-Bares et al. 2004, Kraft et al. 2008, Cornwell and Ackerly 2009, Swenson and Enquist 2009). Trait-based approaches can target the mechanisms of environmental filtering more specifically, but only if critical traits that contribute to fitness, given the abiotic environment, can be identified.

One potential challenge of using trait-based approaches is that multiple traits may be involved in abiotic stress tolerance (Ackerly et al. 2000, Reich et al. 2003, Violle et al. 2007, Cavender-Bares et al. 2009, Losos 2011). There are two reasons why multiple traits may be involved. First, most functional traits are organ-level traits, whereas stress tolerance often depends on the performance of multiple organs (Craine et al. 2012). Second, there could be alternative strategies that are equally effective for tolerating the same stresses (Huner et al. 1993, Schwilk and Ackerly 2001, Chaves et al. 2002, Bailey-Serres and Voesenek 2008). Consider drought tolerance of plants as an example. Organ-level adaptations to drought are found in roots, stems, and leaves (Bréda et al. 2006,

Reich 2014). Furthermore, drought-tolerant plants can be either drought avoiders or drought resistors (Chaves et al. 2002). As a result, there are multiple possible plant phenotypes comprised of different combinations of organ-level traits that confer the same level of drought tolerance. Due to such many-to-one relationships between multi-dimensional trait phenotypes and stress tolerance, variation among species in individual traits may not accurately predict differences in stress tolerance.

Many-to-one relationships can also cause species niche to show a stronger phylogenetic signal compared with the individual traits that underlie the niche (Cavender-Bares et al. 2009, Gerhold et al. 2015). Niche conservatism, the tendency of lineages to retain the same niches over evolutionary time, has been observed in a wide range of taxa (Wiens et al. 2010). Stabilizing selection on niches, which could be driven by lack of sufficient genetic variation to shift niches or competition from co-occurring species filling alternative niches (Ackerly 2003), may cause niches to be conserved within lineages. Conservatism of niches, however, does not require consistent underlying trait combinations. Traits combinations may diverge among close relatives, particularly if divergence allows complementarity in resource use.

A second challenge of using traits-based approaches to test for environmental filtering is that linking functional traits to fitness in a given environment is not possible without experiments. Experiments designed to test for species fitness responses along environmental gradients, with appropriate control of factors beyond the abiotic environment that influence community assembly (e.g., De Steven 1991, Kobe 1999, Emery et al. 2009, Fraaije et al. 2015), provide more direct evidence for environmental filtering than observational approaches based on species abundance or functional traits. Despite this advantage, the experimental approach has been less commonly used to test environmental filtering (Kraft et al. 2015). Here we present an experimental test of

environmental filtering combined with functional trait analyses. Our study system was a group of closely related willow and poplar species (family Salicaceae) native to Minnesota wetlands. Previous studies (Savage and Cavender-Bares 2011, Savage and Cavender-Bares 2012) found Salicaceae species in this region differed in their distributions across a water table depth gradient and drought tolerance traits. These studies provide circumstantial evidence for environmental filtering, mediated by water stresses, along the hydrologic gradient in the Salicaceae species. These studies also found that observed hydrologic niches based on natural abundance patterns of the species were phylogenetically conserved but individual drought tolerance traits were not. These results could be explained if species occupying similar hydrologic niches have different combinations of traits that confer the same environmental tolerance. However, previous studies were not designed to determine how species fitness varies with environment nor how constellations of species-level traits vary with their optimal environments determined by fitness.

This study had two goals. The first goal was to test for environmental filtering and its relative importance in community assembly of the Salicaceae species using a field experimental approach designed to examine species fitness variation across environments (Appendix 1). To address this goal, we transplanted cuttings of 14 species into a series of common gardens established along natural hydrologic gradients, where competition and herbivory were minimized or controlled, and examined responses of species fitness over multiple years. We modeled the optimal water table depth for each species based on their fitness in the field experiment, then compared the water table depth optima to species hydrologic niches determined based on the peak abundance of species in naturally assembled communities. The second goal was to test whether species occupying similar hydrologic niches had different functional trait combinations that conferred similar

environmental tolerances (assessed using species fitness variation across environments). We analyzed a suite of functional traits associated with water transport and water stress tolerance, and compared phylogenetic signals in the traits with species hydrologic niches and integrated stress tolerance. To address this goal, we compiled a data set of nine functional traits, which included measurement of three traits novel to this study, each with known importance for tolerating drought or flooding. We then tested three predictions: i) traits do not all co-vary with each other in a coordinated manner, ii) if (i) is upheld, species occupying similar hydrologic niches have different combinations of traits; and iii) species hydrologic niches and integrated water stresses tolerance are phylogenetically conserved, but individual traits are not.

Materials and Methods

Study site

The study was conducted at Cedar Creek Ecosystem Science Reserve (CCESR) in central Minnesota, U.S. (45° 24' 0" N, 93° 12' 0" W). The local climate is continental, with a mean annual average daily temperature of 6.3 °C and a mean annual total precipitation of 776 mm based on records from 1963 to 2012 (CCESR online database). The soils are sandy and poor in nutrients (Grigal and Homann 1994). The reserve has a mosaic landscape consisting of numerous patches of prairie, wooded uplands, and wetlands (Appendix 2). With the exception of several bogs, the majority of wetlands at CCESR share the same regional water table. Due to this feature of hydrology, most wetlands at CCESR have synchronized seasonal water table depth variation, which peaks in early spring due to snowmelts then gradually declines as the growing season proceeds.

Hydrologic niches of the Salicaceae species

Fourteen native Salicaceae species, including 13 willows (genus *Salix*) and one poplar (genus *Populus*) naturally occur within CCESR (Appendix 1). Savage & Cavender-Bares (2012) found using a field survey that these species had differential distributions along water table depth gradients. Briefly, they established 50 10m by 30m plots at randomly chosen locations within CCESR and three preserves nearby (Helen Allison Savanna Scientific and Natural Area, 45° 22' 48" N, 93° 10' 48" W; Boot Lake Scientific and Natural Area, 45° 20' 24" N, 93° 7' 12" W; and Carlos Avery Wildlife Management Area, 45° 20' 42" N, 93° 01' 12" W), and measured total basal area of the species and monthly water table depth in these plots from 2007 to 2009. Two metrics of hydrologic niche were calculated based on species abundances and water table depth in the plots, which were mean water table depth averaged across all plots weighted by species abundances in each plot during the wettest month (May, WT_{wet}) and the driest month (August, WT_{dry}) during growing seasons (Appendix 1). WT_{wet} ranged from -102 to 7 cm across species; and WT_{dry} ranged from -110 to 0 cm (negative values indicate the water table was below the ground level; positive values indicate the water table was above the ground level). Due to the synchronization of water table depths in the wetlands at the study area, the two hydrologic niche metrics were highly correlated among species, with a Pearson correlation coefficient of 0.94.

Field experiment

The experiment included 40 common gardens located at ten different sites at CCESR (Appendix 2). Each site had a natural wetland, around which four common gardens, separated into two pairs, were built. The two gardens in each pair were located at the two ends of a transitional zone connecting the wetland to its adjacent upland (Appendix 2). Twenty-eight cuttings, two from each species, were planted in each garden. The two

conspecific individuals were always planted next to each other; and locations of conspecific pairs were randomly arranged within gardens. Whenever enough plant material was available, we planted four conspecific cuttings of the same genotype into a single common garden pair to avoid possible confounding effects between genotype and water table depth on experimental plants. We were able to control for genotype in this manner in 87% of experimental plants. To reduce competition between experimental plants, as well as between experimental plants and naturally occurring vegetation, adjacent cuttings were planted 1.4 m away from each other; and a piece of 1m by 1m landscape fabric was installed beneath each plant to inhibit the growth of native vegetation. To exclude large mammalian herbivores (e.g., deer) the gardens were surrounded by metal fences 2.5m in height.

The experimental design also includes a cage treatment nested within gardens as a part of a separate study on insect herbivory (Appendix 2). During the growing season, one individual of each conspecific pair was enclosed in a sham cage, and the other individual was enclosed in a real cage. The cages were made from fine white polyester mesh and had a cuboid shape of 0.5m by 0.5m by 1.2 m. Some cages were extended to 2.4 m tall during the second year to accommodate two fast-growing species (*S. eriocephala* and *S. lucida*). The designs of real and sham cages were the same, except that sham cages had openings that allowed insects to access experimental plants.

Experimental plants were propagated from branch cuttings collected from natural populations of the species at CCESR and three preserves nearby (described previously). In 2009 and 2010, 13 to 31 maternal plants per species were sampled to capture a wide range of genotypic variation within species. Cuttings were soaked in tap water for a few days to root. Rooted plants were grown in individual pots in a greenhouse room at University of Minnesota, St. Paul. In fall 2010, these plants were transplanted into

common gardens after a several-day-long adjustment period in the field during which they were kept in pots.

Two plant fitness components, survival and basal area, were measured at the end of the 2012 growing season. The water table depth in each garden was measured bi-weekly during the 2012 growing season from a well installed at the center of the garden, using a steel tape measure. The wells were made by attaching a PVC well point (Campbell Manufacturing, LLC) to a section of PVC pipe of the same inner diameter (5cm), and were installed 0.6 m and 1.5 m below ground in wetland and upland gardens respectively. The well points had a closed bottom and fine slots on their walls that allowed water to move in and out of the wells. Besides drought and flooding, nutrient availability can also vary along hydrologic gradients (Silvertown et al. 2015) and influence plants fitness; therefore we measured nitrogen availability in common gardens as a covariate of plant fitness. Specifically, plant-available soil nitrogen was measured using ion exchange resin bags at one randomly chosen pair of gardens per site during the peak of 2011 growing season (July to August). The bags were placed 10 cm below the soil surface, extracted with 2M KCl, and analyzed for inorganic nitrogen using a colorimetric method (Riggs et al. 2015).

Collection of functional traits data

We measured three traits (lenticel density on a first year branch, lenticel density on the main stem, and stem specific hydraulic conductivity) and compiled data on six other traits from previous studies of a larger set of Salicaceae species. The traits were chosen for their known associations with drought or flooding tolerance. Below, we describe methods used for collecting the three traits novel to this study and provide references for the trait data that were published.

Lenticel densities —In 2014, we measured lenticel densities on the main stem and on a first-year branch of each experimental plant. We counted the number of lenticels on a 3 cm-long section of the main stem and a randomly selected first year branch. Lenticel densities were calculated as number of lenticels per cm^2 of surface area of the stem or of the branch, assuming the stem had a cylinder shape and the branch a frustum shape. The sampled main stem sections were right above water tables (if the plant was in standing water) or the highest adventitious roots (if the plant had been flooded earlier in the growing season) or ground levels. These locations were chosen because lenticels right above water tables are known to be entry points of oxygen into flooded plants (Armstrong 1968).

Stem specific conductivity —We estimated stem specific conductivity based on vessel distributions in five or six two-year-old cuttings from each species. These cuttings were grown in a greenhouse room in Franklinville, New York, U.S. (42° 20' 24" N, -78° 27' 36" W) and experienced local climate conditions (Savage and Cavender-Bares 2013). Cross sections were made from one first-year branch per plant. Vessel diameters and sapwood areas were measured by taking microscopic photos, which were then analyzed using Image J (Schneider et al. 2012). Stem specific conductivity was calculated from vessel diameters following Tyree & Ewer (1991). Average vessel diameters of the species were reported in Savage and Cavender-Bares (2013).

Trait data compiled from previous studies—Turgor loss point, stomatal pore index, root elongation rate, wood density, maximum photosynthetic rate, and stomatal conductance were collected from natural populations in central Minnesota and have been reported in Savage (2009) and Savage & Cavender-Bares (2012).

Data analysis

All analyses were done using R (R Core Team 2016).

Aster fitness models —Species fitness responses along experimental water table depth gradients were analyzed using aster fitness models (Geyer et al. 2007, Shaw et al. 2008). Different fitness components, such as survival and growth, are usually analyzed separately for they do not follow the same probability distribution. Such separate analyses may yield contrary results, making it difficult to conclude about the overall responses of species to environmental gradients. Aster fitness models analyze multiple fitness components jointly. In an aster model, the "response" is a vector of random variables, each of which is a fitness component modeled by its appropriate statistical distribution. Furthermore, these response variables are modeled as non- independent from each other because fitness components expressed later in life history depend on those expressed earlier (e.g., a plant will have a biomass of zero if it is dead). Parameters associated with all fitness components were estimated jointly using Maximum Likelihood.

To test effects of species, water table depth in the common gardens, and their interaction on plant fitness, we constructed four alternative aster models of all experimental plants (Appendix 5) and compared these models using Akaike Information Criterion (AIC) (Akaike 1974). The four models differed from each other by a) the shape of species fitness response to water table depth, and b) whether a species by water table depth interaction was included or not. Species fitness was modeled either as a linear or a quadratic function of water table depth. Along water table depth gradients, intensities of two different stresses, drought and flooding, change in opposite directions. If species were sensitive to only one type of stress, their fitness would show monotonic responses across the water table depth gradient, which were modeled by linear functions. If species were sensitive to both stresses, their fitness would show hump-shaped responses, which were modeled by quadratic functions. To account for the seasonal variation in water table

depth, we constructed two sets of aster models using growing season maximum and minimum water table depth in common gardens respectively (i.e. eight models total). Three covariates were included in all models, which were common garden site, species by cage treatment interaction, and the residual of soil nitrogen availability after regressing it on water table depth (we used residuals instead of the original values because soil nitrogen availability was correlated with water table depth).

To infer the relative importance of environmental filtering to the determination of natural distributions of the Salicaceae species, we predicted the water table depth where each species reached its highest fitness based on the best aster model selected by AIC. We then performed a regression of species natural hydrologic niche on their optimal water table depth predicted by the best aster model. A higher coefficient of determination (r^2) would indicate a stronger effect of environmental filtering on species distributions. All aster model analyses were performed using the aster package (Geyer *et al.*, 2007).

Functional trait analyses —We first performed univariate regressions of hydrologic niche and optimal water table depth on individual traits to test whether traits were associated with drought or flooding tolerance in the Salicaceae species as we predicted based on the literatures (Appendix 4). To test the first two predictions of the second goal of the study- which are: i) traits do not all co-vary with each other in a coordinated manner, and ii) species occupying similar hydrologic niches have different combinations of traits - we tested pair-wise correlations between traits, and performed a principal component analysis (PCA) of traits. Additionally, to examine whether multi-trait phenotype could predict species hydrologic niche or optimal water table depth better than individual traits, we performed regressions of hydrologic niche and optimal water table depth on principal components of the trait PCA.

To test the last prediction of the second goal of the study-which is: hydrologic niche was phylogenetically conserved, but individual traits were not- we analyzed phylogenetic signals in traits, principal components of the trait PCA, hydrologic niche, and water table depth optima using the Blomberg's K statistic (Blomberg et al. 2003). We used a published phylogeny constructed from five chloroplast barcode genes and a nuclear alcohol dehydrogenase gene (Savage & Cavender-Bares, 2012) and compared observed K values against two null distributions: a random null distribution calculated by randomly swapping trait values across the tips of the phylogeny and repeated 10000 times, and a Brownian Motion null distribution calculated by simulating trait evolution using the sim.char function in the GEIGER package (Harmon et al. 2008) and repeated 10000 times.

To correct for non-randomness in trait data due to phylogenetic relatedness among species, we re-run the above-mentioned regressions using phylogenetically independent contrasts of the traits calculated by the pic function in the ape package (Paradis et al. 2004). We did this only if both variables in the regression showed significant phylogenetic signals against random null distributions and no significant difference from Brownian motion null distributions. We did not perform phylogenetically corrected PCA (pPCA), because the majority of traits did not show significant phylogenetic signal against random null distributions, thus violating the assumption of pPCA that traits included in pPCA analysis should follow a multivariate Brownian motion model (Revell 2009).

Results

Aster fitness models

In 2012, growing season maximum and minimum water table depth in the common gardens ranged from -120.1 to 25.4 cm and from -162.6 to 11.4 cm respectively (Appendix 2), covering the complete range of species' natural hydrologic niches. The two water table depths were strongly correlated with each other (with a correlation coefficient of 0.92); and AIC comparisons among aster models constructed using the two water table depth predictors showed similar results. Consequently, we report the results of the models constructed using growing season maximum water table depth in the main text only and used these results for subsequent analyses. The results of the models constructed using growing season minimum water table depth are reported in Appendix 6 & 7.

The best aster model included a quadratic, concave function of water table depth and a species by water table depth interaction effect (Appendix 5). Optimal water table depth of the species predicted by the best aster model ranged from -89.5 to 2.4 cm, and was significantly correlated with species natural hydrologic niche ($P<0.0001$, $r^2=0.75$; Figure 1.2c).

Functional trait analyses

Seven out of nine traits showed significant correlations with hydrologic niche and four had the same signs as expected (Appendix 4). Specifically, turgor loss point significantly increased and root elongation rate, stomatal pore index, and stem specific conductivity significantly decreased with hydrologic niche. By contrast, lenticel density on the young branch, maximum photosynthetic rate, and stomatal conductance significantly decreased with hydrologic niche, which was the opposite of our prediction. Correlations between individual traits and water table depth optima showed a similar pattern as that of natural hydrologic niche, except that stem specific conductivity had a negative but insignificant correlation with water table depth optima (Appendix 4). Three

out of four published traits (turgor loss point, root elongation rate, stomatal pore index) showed the same correlations with hydrologic niche as previously found in a larger set of Salicaceae species (Savage & Cavender-Bares 2012).

Although seven traits showed a significant correlation with the hydrologic niche, each trait was only correlated with two to four other traits (Figure 1.2a). On a biplot of the first and second principal components of the trait PCA (Figure 1.2b), traits clustered into two groups: traits in the first group, including stomatal pore index, root elongation rate, wood density, and lenticel density on main stems, were predominantly located in the lower right quadrant, except that turgor loss point was in the upper left quadrant. Traits from the second group, including photosynthetic rate, stomatal conductance, stem specific conductivity, and lenticel density on young branches, were located in the upper right quadrant. The vectors of two groups of traits pointed in perpendicular directions, indicating that traits within groups were more correlated with each other than with traits in the other group. Three upland species, *S. humilis* (HUM), *S. interior* (INT), and *P.deltoides* (DEL), were scattered in different parts of the plot, indicating they had different trait combinations. The first principal component, accounted for 48% of the total variance in the data, was negatively correlated with both hydrologic niche and optimal water table depth ($P<0.0001$, $r^2=0.74$ and $P=0.005$, $r^2=0.49$, respectively; Figure 1.2d, 1.2e); none of the rest principal components was not correlated with either hydrologic niche or optimal water table depth. Subsequently, we only tested the phylogenetic signal in the first principal component.

The observed K values of species hydrologic niche and the first principal component of the trait PCA were significantly higher than the means of random null distributions ($P<0.05$), although none of the individual traits did (Figure 1.3). The observed K values of water table depth optima, root elongation rate, and stomatal pore index were

marginally significantly higher than the means of random null distributions (P <0.10). The observed K values of turgor loss point, wood density, and photosynthetic rate were significantly lower, and lenticel density on main stems marginally significantly lower than the means of Brownian motion null distributions. Since both species hydrologic niche and the first principal component showed significant phylogenetic signals, we re-ran regressions between the two using phylogenetically independent contrasts and found similar results as we did for the non-phylogenetically controlled regression (r^2=0.52 *P*= 0.005).

Discussion

We found fitness variation among 14 Salicaceae species, experimentally planted along a water table depth gradient in the absence of competition and herbivory. This result provides strong evidence that environmental filtering can drive differential distributions among species along an important environmental gradient. Some of the functional traits linked to water transport efficiency and water stress tolerance varied independently from each other, which we hypothesize allows species occupying similar hydrologic niches to have different trait combinations. As a result, individual traits were less phylogenetically conserved than species hydrologic niches or their integrated function determined by multiple traits associated with water transport and water stress tolerance.

A controlled experimental revealed the critical role of environmental filtering in community assembly

The importance of environmental filtering in the community assembly of the Salicaceae species was shown by the high coefficient of determination (r^2) in the

regression of species hydrologic niche determined by natural abundance on optimal water table depth determined by species fitness in the field experiment (Figure 1.2c). Environmental filtering was caused by both drought and flooding stresses, as species fitness showed a quadratic, concave response along experimental hydrologic gradients in the best aster model (Figure 1.1, Appendix 5). These results corroborated conclusions of a previous study that found variation in species abundance and functional traits along hydrologic gradients in naturally occurring Salicaceae populations (Savage & Cavender-Bares 2011).

A comparison of this study to other experimental works shows that different processes may dominate community assembly of plant species along hydrologic gradients. For instance, Emery et al. (2009) investigated annual plant communities in California vernal pools and found similar results as this study: environmental filtering caused by hydrologic stresses was the dominant process determining species distributions. In contrast, Fraaije et al. (2015) found that among riparian species distributed along stream hydrologic gradients in the Netherlands, dispersal constraint played a more important role than environmental filtering. Although these studies found similar patterns of hydrologic niche segregation, experimental tests helped reveal the underlying differences in community assembly processes.

Many-to-one relationships between multi-trait phenotypes and function

Several results supported the hypothesis that species could have different trait combinations but similar water transport capacity and water stress tolerance that allow them to occupy a similar hydrologic niche. First, only 13 out of 36 pair-wise correlations between traits were statistically significant (Figure 1.2a). Second, three upland species had different trait combinations, as shown in the biplot of the traits PCA (Figure 1.2b).

Finally, while both the hydrologic niche and the first principal component of the traits PCA were phylogenetically conserved, individual traits were not (Figure 1.3b).

One pattern that emerged from trait analyses was that while wetland species had similar combinations of all traits, upland species had contrasting combinations of trait values. This pattern may reflect differences in the drought tolerance strategies of upland species (Figure 1.3a). Traits examined in this study could be separated into two groups based on their correlations, so that traits within groups were more correlated with each other than with traits in the other group (Figure 1.2a, 1.2b). One group of traits included turgor loss point, stomatal pore index, root elongation rate, wood density, and lenticel density on main stems; the other group consisted largely of traits related to gas exchange and hydraulic conductance. One of the upland species, *S. humilis* had similar values to wetland species in the second group of traits, but more extreme values in the first group of traits. In contrast, *S. interior* had similar values to wetland species in the first group of traits, but more extreme values in the second group of traits, which are related to gas exchange rates and hydraulic conductance. High gas exchange rates and hydraulic conductance allow plants to respond quickly to rainfall events, taking advantage of temporarily available water supply (Maherali and DeLucia 2000, Maherali et al. 2004). Compared to *S. humilis*, *S. interior* may have a drought-avoidance rather than a drought-resistance strategy.

In a greenhouse dry-down experiment, Savage & Cavender-Bares (2011) also found that three wetland Saliaceae species (*S. bebbiana*, *S. discolor*, *S. petiolaris*), all with intermediate habitats along the hydrologic gradient, showed different drought tolerance mechanisms. Together, these functional studies reveal important variation in drought tolerance strategies among the Salicaceae species. Divergence in ecological strategies among species co-occurring in the same environments has been found in various plant

communities (Goulden 1996, Hirose and Werger 1995, Hacke et al. 2000). Such many-to-one relationships between phenotypes and function may promote coexistence by allowing differentiation in resource use strategies among species. The fact that hydrologic niche was phylogenetically conserved, while the traits were not, indicate that the conservatism in hydrologic niche is unlikely to be caused by evolutionary constraints in individual traits. Instead, stabilizing selection driven by competition may promote niche conservatism, while allowing trait combinations to diverge among close relatives.

Many-to-one relationships between multi-trait phenotypes and function present unique challenges for the use of trait-based or phylogenetic approaches to test community assembly processes. First, such relationships require incorporating multiple traits for predicting individual performance based on traits (Marks and Lechowicz 2006, Sterck et al. 2011, Sterck et al. 2014, Kraft et al. 2015b, Laughlin and Messier 2015, Schroeder-Georgi et al. 2016). In this study, although seven out of nine traits showed a significant correlation with hydrologic niche, these traits all explained less variation in the hydrologic niche than the first principal component of traits. Second, many-to-one relationships between trait combinations and stress tolerance could complicate the interpretation of community phylogenetic patterns (Cavender-Bares et al. 2009, Gerhold et al. 2015). When closely related species have evolved different strategies to solve the same ecological problems (e.g., Schwilk and Ackerly 2001, Agrawal and Fishbein 2006), they may diverge in individual traits while maintaining similar ecological niches, as shown in this study (Figure 1.3), which may cause phylogenetic clustering in communities without phenotypic clustering.

Conclusions

Our experimental test of fitness differences among willow and poplar species across hydrologic gradients highlights the importance of environmental filtering in community assembly. Our controlled field experimental approach allowed us to detect variation among species in growth and mortality in response to the environment in the absence of possible confounding effects of dispersal and biotic interactions. This study reveals that species with contrasting trait combinations have similar hydrologic niches and similar water transport efficiency and water stress tolerance. Due to the many-to-one relationship between trait combinations and function, while species hydrologic niche was phylogenetically conserved, individual traits underlying hydrologic niche were not. The phylogenetic conservatism of the niche, in contrast to the lability of individual functional traits, suggests that species hydrologic niches may be under stabilizing selection and not constrained by individual traits.

Figure 1.1 Species fitness response along the experimental hydrologic gradient predicted by the best aster fitness model. Biomass at the end of the second growing season was used as a surrogate for fitness. A predicted biomass of zero or less indicates that the species could not survive at the given water table depth.

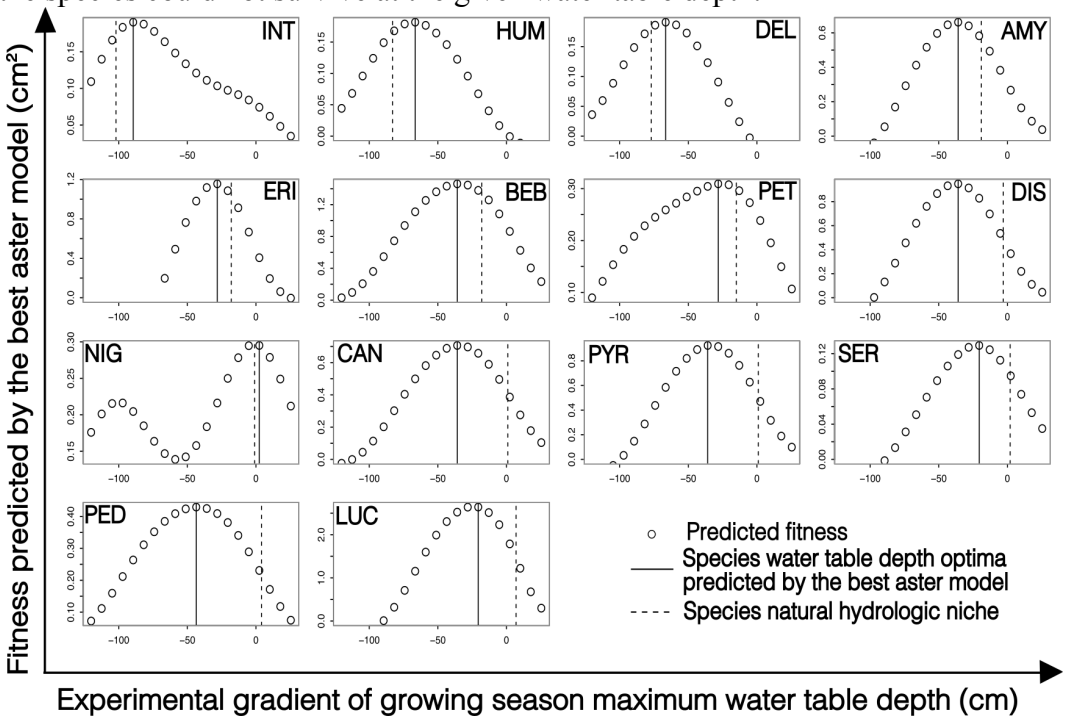


Figure 1.2 (a) Correlations among traits. Black and grey lines connecting two traits represent positive and negative correlations, respectively. The intensity of the colors indicates the strength of the correlations. Only significant and marginally significant correlations (P<0.1) are shown. Note not all traits were correlated with each other. **(b) A biplot showing the scores and loadings on the first two principal components from a principal component analysis (PCA) of traits.** The traits were separated into two groups. The first group of traits, including A_{max}, g_s, LD_y, and K_s, are associated with gas-exchange and hydraulic conductance. The second group of traits, including TLP, SPI, RER, WD, and LD_b, mostly are morphological traits related to water stresses tolerance. Note that the two groups of traits point in perpendicular directions, indicating traits were more correlated with members within the same group than with traits in the other group. Also note that the three upland species, *S. interrior* (INT), *S. humilis* (HUM), and *P. deltoids* (DEL), are located in different places in the biplot, indicating they had different trait combinations. **(c) Species natural hydrologic niche was significantly correlated with water table optimum predicted by the best aster fitness model** (P<0.0001, r^2=0.75). The solid line is the least square regression line; the dashed line is the 1 to 1 line. (d) Species natural hydrologic niche was significantly correlated with the first principal component of the trait PCA (P<0.0001, r^2=0.74). (e) Species water table depth optimum was significantly correlated with the first principal component of the trait PCA (P=0.005, r^2=0.49).

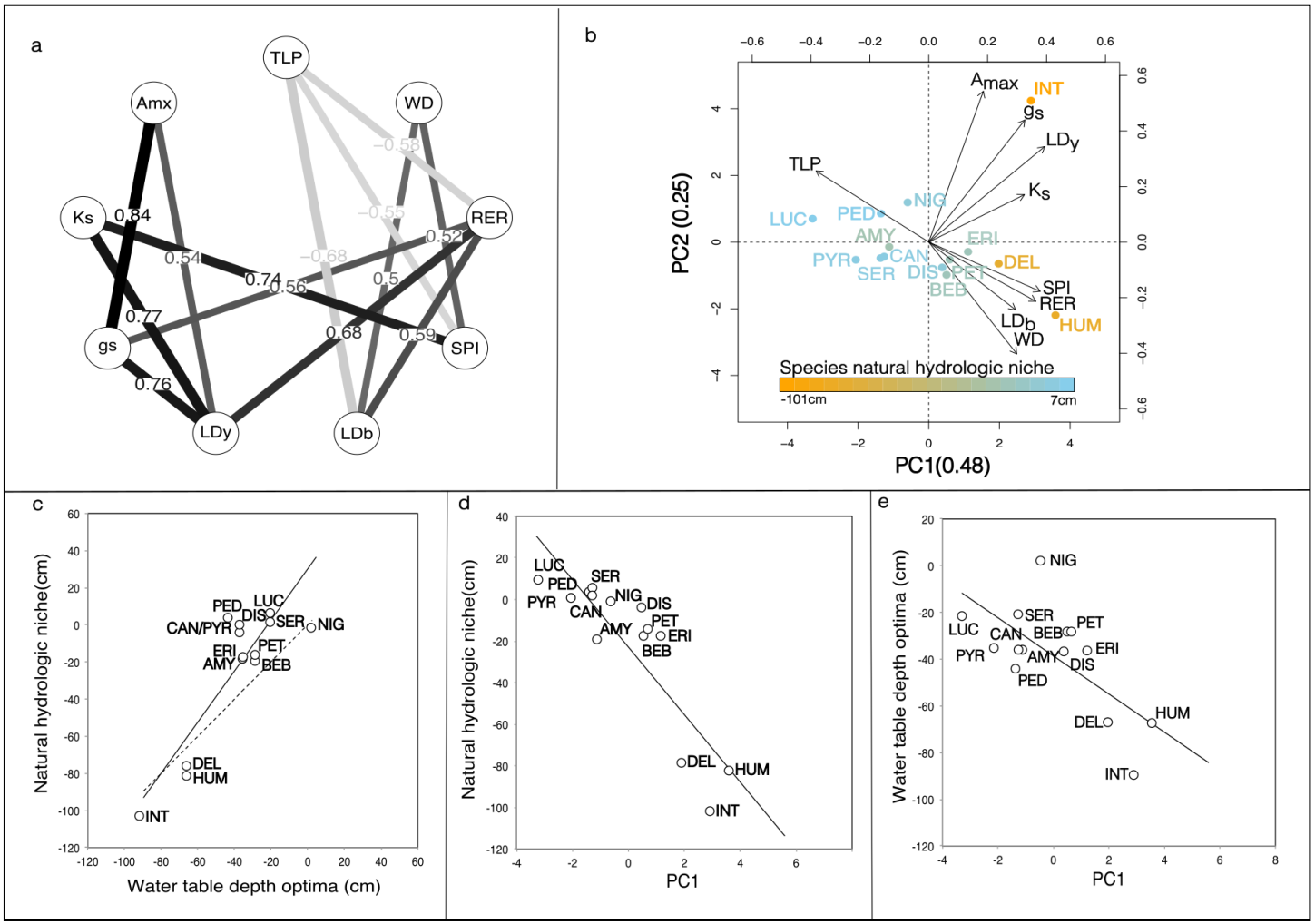
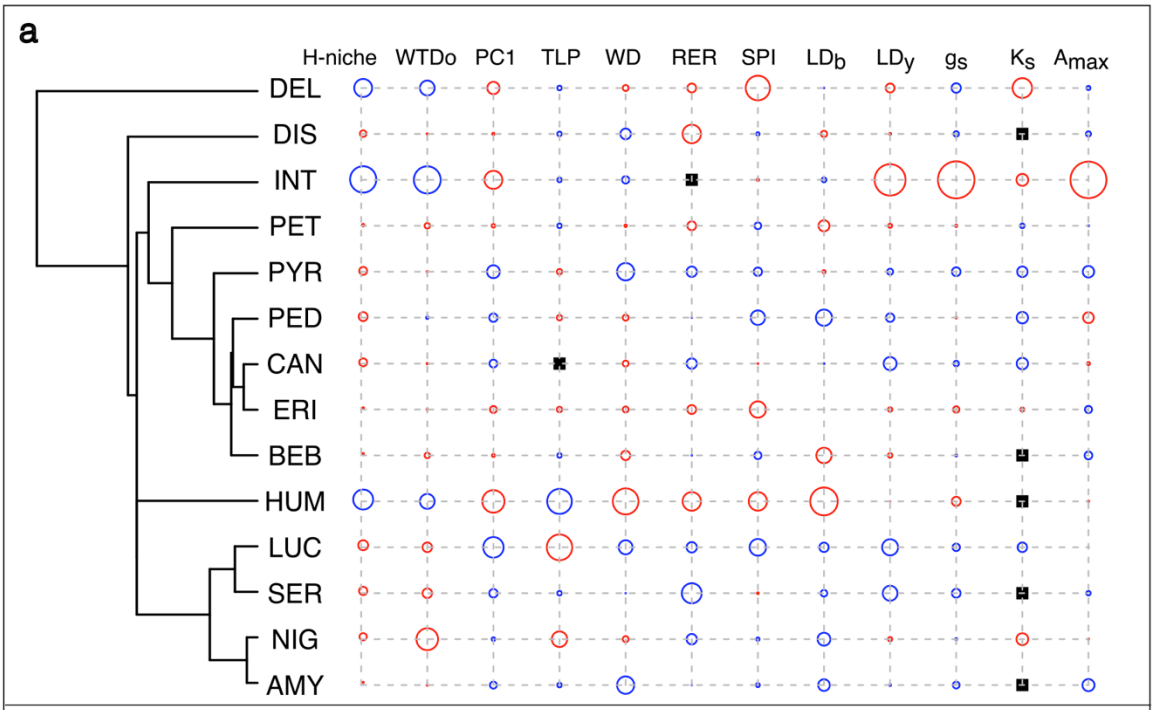

a
TLP
Amx
WD
Ks
RER
gs
SPI
LDy
LDb
0.84
0.54
0.74
0.56
0.77
0.76
0.68
0.59
0.5
0.52
b
PC2 (0.25)
PC1(0.48)
Amax
INT
gs
LDy
TLP
Ks
NIG
LUC
PED
AMY
ERI
PYR
CAN
SER
DIS
PET
BEB
DEL
SPI
RER
LDb
HUM
WD
Species natural hydrologic niche
-101cm
7cm
c
Natural hydrologic niche(cm)
Water table depth optima (cm)
PED
LUC
DIS
CAN/PYR
SER
NIG
ERI
PET
AMY
BEB
DEL
HUM
INT
d
Natural hydrologic niche(cm)
PC1
LUC
PED
SER
PYR
NIG
DIS
CAN
PET
AMY
ERI
BEB
DEL
HUM
INT
e
Water table depth optima (cm)
PC1
NIG
SER
PET
LUC
CAN
BEB
ERI
PYR
AMY
DIS
PED
HUM
DEL
INT

Figure 1.3. **(a) Distributions of species hydrologic niche (H-Niche), water table depth optimum predicted by the best aster fitness model (WTD_o), the first principal component of the traits PCA (PC1), and individual traits across the Salicaceae phylogeny.** Circles indicate normalized trait values of the species (we normalized trait values by first subtracting the mean of the trait, then dividing the remainder by the standard deviation of the traits). Red and blue circles indicate positive and negative values, respectively; and diameters of circles indicate absolute values of normalized traits. Black squares indicate missing data. **(b) Results of Blomberg's K statistic tests. Characters' observed K values were compared to random and Brownian motion null distribution.**



b

K_{obs}	1.18	0.55	0.65	0.26	0.27	0.51	0.52	0.46	0.50	0.60	0.59	0.37
$P(K_{obs}>K_{Rand})$	0.01	0.09	0.02	ns	ns	0.06	0.09	ns	ns	ns	ns	ns
$P(K_{obs}<K_{BM})$	ns	ns	ns	0.003	0.005	ns	ns	0.09	ns	ns	ns	0.03

Can insect herbivory promote beta diversity among host plant communities? A field experimental test in closely related Salicaceae species across hydrologic gradients

Summary

Although insect herbivory is known to promote plant alpha diversity within communities, its effect on plant beta diversity remains under-investigated. In this study, we tested the hypothesis that insect herbivory promotes habitat segregation along hydrologic gradients among 14 closely related willow and poplar (Salicaceae) species using a field experimental approach. Cuttings of the species were transplanted into a series of common gardens established across natural water table depth gradients at the Cedar Creek Ecosystem Science Reserve. We measured the effect of herbivory on plant growth using a cage treatment nested within the gardens. We also measured five leaf traits associated with secondary chemistry and nutrient quality, and tested phylogenetic signals in these traits.

Insect herbivory reduced plant growth, was different between species, and varied across the water table depth gradient in a hump-shaped manner. However, herbivory did not promote habitat segregation among the host plant species because there was no interaction effect between species and water table depth on either herbivory or on the cage treatment effect on plant growth. Variations in leaf traits could partially explain the variation in herbivory between species but not the variation across hydrologic gradients. Closely related species did not share similar defense traits: although secondary metabolite richness was phylogenetically conserved, concentrations of defense compounds and nitrogen were not. Our study indicates that insect herbivory did not promote beta diversity among the Salicaceae communities. On the other hand, closely related Salicaceae species are known to share similar hydrologic environments; therefore, the

dissimilarity in their defense chemistry might promote the coexistence among close relatives within local communities through density-dependent effects.

Key words: beta diversity, habitat segregation, herbivory, plant secondary metabolites, plant defenses, phylogenetic signals, hydrologic gradients, Salicaceae (willow and poplar).

Introduction

Beta diversity, the variation in species composition across environmental gradients (Whittaker 1960, 1972), is an important component of species diversity that influences diversity patterns at medium to large spatial scales (Qian & Ricklefs 2007, Kraft et al. 2011). Environmental filtering and dispersal are two known drivers of plant beta diversity (Tuomisto et al. 2003, Qian 2009). Besides these processes, biotic interactions can also influence plant beta diversity, as they are often context-dependent and vary with abiotic environments (e.g., Jabot & Bascompte 2012, Mclntire & Fajardo 2013).

Among biotic interactions, herbivory is a particularly important interaction for plants. Herbivory can affect not only the fitness of individual plants (Crawley 1989), but also plant community assembly through mechanisms such as the Janzen-Connell effect (Comita et al. 2014) and the competition-defense trade-off (Viola et al. 2010). Although the effect of herbivory on the alpha diversity of plant communities is well studied, its impact on plant beta diversity remains under-investigated (Louda 1989; Maron & Crone 2006). The impacts of herbivory damages on plant performance can vary along environmental gradients and limit plant distributions (Maron & Crone 2006). However, most previous studies focused on a single plant species; therefore they did not directly address the question whether herbivory influences plant beta diversity or not.

A few studies have compared the effect of herbivory on plant performance across environmental gradients between multiple co-occurring species; the results are mixed (e.g., Fine et al. 2004, 2006, Brenes-Arguedas et al. 2009). With the limited number of

studies, it is still difficult to make generalizations about the impact of herbivory on plant beta diversity. Furthermore, most previous studies were conducted in tropical forests (but see Louda 1982, 1983); it is still largely unknown whether or not herbivory can promote habitat differentiation among plant species in temperate regions, where the intensity of herbivory is considered to be lower than in the tropics (Coley & Barone, 1996; Mittelbach et al., 2007)

Recently, there is an increasing interest in using phylogenetic relatedness to predict plant-herbivore interactions (Pearse & Hipp, 2009; Ness *et al.*, 2011; Castagneyrol *et al.*, 2014; Lind et al. 2015). Central to these studies is the hypothesis that insect herbivores prefer to feed on closely related plant species. Empirical studies indicate that this hypothesis holds true when herbivore preference is examined among plant species over a wide range of taxonomical groups (Novotny & Basset, 2005; Weiblen *et al.*, 2006; Dyer *et al.*, 2007). However, the hypothesis does not necessarily hold true when herbivore preference was compared among host plant species within the same families or genera (e.g., Becerra 1997). Theoretically, herbivores should prefer closely related host plant species if they select hosts based on plant traits that are phylogenetically conserved, and feed on distantly related species if they select hosts based on plant traits that are phylogenetically labile.

Plant secondary metabolites (SMs) have long been recognized playing an important role in plant-herbivore interactions (for reviews, see Berenbaum & Zangerl 1997; Futuyma & Agrawal 2009; Agrawal & Weber 2015). These compounds can deter feeding

in generalist herbivores, but may have little effect on or even stimulate feeding in specialist herbivores. Plant species within the same family or genus often share a group of SMs of similar chemical structures that seldom occur outside the lineage. Such phylogenetic signals in secondary chemistry are hypothesized to be a driver for the specialization of insect herbivores to closely related host plant species (Ehrlich & Raven 1964). On the other hand, the compounds shared by con-familial or congeneric plant species are not exactly equivalent: they have the same core structures but different side chains, therefore differential biochemical properties. Insect herbivores can show differential responses to different compounds within the same host plant family or genera, which could result in specialization to individual host plant species or populations (Berenbaum 1995).

In this study, we examined the effect of insect herbivory on the beta diversity among communities of closely related willow and poplar (Salicaceae) species native to Minnesotan wetlands, and phylogenetic signals in secondary metabolites of the species. The Salicaceae species provide a very suitable system to address these questions for three reasons. First, the species showed differential distributions across hydrologic gradients at our study site (Savage & Cavender-Bares 2011, 2012). Second, species in the Salicaceae family are known to host a variety of insect herbivores (Volf et la. 2015a, b). Last, the Salicaceae species have two groups of secondary metabolites, condensed tannins and phenolic glycosides, with known effects on insect herbivory (see Materials and Methods for details).

To examine the effect of herbivory on beta diversity among Salicaceae communities, we transplanted cuttings of 14 Salicaceae species into a series of common gardens established across natural hydrologic gradients in the field. We examined the effect of herbivory on plant growth using a cage treatment nested within the gardens. We measured growth, the amount of herbivory damages, and foliar concentrations of nitrogen, carbon, and the two groups of defense compounds to address the following three questions. First, did the effect of herbivory on plant growth, measured by the cage treatment effect, vary across hydrologic gradients differentially among the Salicaceae species? If so, this would provide evidence for insect herbivory promoting beta diversity among the Salicaceae communities. Second, was the variation in herbivory effect on plant growth caused by variations in the amount of herbivory damages and the leaf traits? Last, did the leaf traits showed phylogenetic signals?

Materials and Methods

The field site and the Salicaceae species

The study was conducted at Cedar Creek Ecosystem Science Reserve (CCESR) in central Minnesota, U.S. (45° 24’ 0” N, 93° 12’ 0” W). The local climate is continental. The mean annual daily average temperature was 6.3 °C, and the mean annual total precipitation was 776 mm over the last five decades (Seeley, 2012). The soils are sandy and nutrient-poor (Grigal & Homann, 1994). The reserve has a mosaic landscape consists of numerous patches of prairie, wooded uplands, and wetlands. 14 native Salicaceae

species, including 13 willows (genus *Salix*) and one poplar (genus *Populus*) co-occur within the reserve.

Species in the Salicaceae family have two groups of secondary metabolites that can influence herbivory resistance: phenolic glycosides and condensed tannins. Phenolic glycosides (PGs) are unique to the Salicaceae family. They can cause degenerative mid-gut lesion in insect herbivores (Lindroth 1988; Lindroth & Peterson 1988). PGs effectively deter the feeding and reduce the performance of generalist herbivores (Boeckler et al. 2011), but their effects on the performance of specialist herbivores range from negative (Lindroth & Hwang 1997; Young et al. 2010), neutral (Matsuki & MacLean Jr. 1994), to positive (Orians et al. 1997; Boeckler et al. 2011; Volf et al. 2015a; 2015b). In contrary, condensed tannins (CTs) are widely distributed in plants and generally have little effect on the feeding preference and performance of insect herbivores (Barbehenn & Constabel 2011; Madritch & Lindroth 2015; but see Bryant et al. 1993; Ayres et al. 1997).

Field experiment

The experiment included 40 common gardens located at ten different sites at CCESR. Each site had a natural wetland, around which we built four common gardens. The four gardens were separated into two pairs. Each pair included an upland garden and a wetland garden, which were located at the two ends of a transitional zone connecting the wetland to its adjacent uplands. 28 cuttings, two from each of the 14 species, were

planted into each garden. The two conspecific individuals were always planted next to each other. Locations of conspecific pairs were randomly arranged within gardens. Whenever enough plant materials were available, we planted four conspecific cuttings of the same genotype into a single common garden pair to avoid possible confounding effects between genotype and water table depth on experimental plants. We were able to control for genotype in this manner for 87% of experimental plants. During the 2011 and 2012 growing seasons, we installed a real and a sham cages around the two individuals of each conspecific pairs. The cages were made from fine white polyester mesh and had a cuboid shape of 0.5m by 0.5m by 1.2m. The height of cages was extended to 2.4 m during the second growing season to accommodate two fast-growing species (*S. eriocephala* and *S. lucida*). The design of real and sham cages were the same, except that sham cages had openings to allow insects accessing the plants. To reduce competition between experimental plants, adjacent experimental plants were planted 1.4 m away from each other. A piece of 1m by 1m landscape fabric was installed beneath each plant to inhibit the growth of native vegetation. The gardens were surrounded by metal fences 2.5 m in height to exclude large mammalian herbivores (e.g. deer).

Experimental plants were propagated from stem cuttings collected in natural populations of the Salicaceae species at CCESR and three preserve areas nearby (Helen Allison Savanna Scientific and Natural Area, 45° 22’ 48” N, 93° 10’ 48” W; Boot Lake Scientific and Natural Area, 45° 20’ 24” N, 93° 7’ 12” W, and Carlos Avery Wildlife Management Area, 45° 20’ 42” N, 93° 01’ 12” W). In 2009 and 2010, 13 to 31 maternal

plants per species were sampled to capture a wide range of genotypic variations within the species. Cuttings were soaked in tap water for a few days to root. The rooted plants were planted into individual pots and kept in a greenhouse room at University of Minnesota, St. Paul. In fall 2010, the cuttings were transplanted to the common gardens after a several-day-long adjustment period in the field during which they were kept in pots.

The basal area of experimental plants was measured at the beginning and end of 2012 growing season. In August 2012, we sampled 20 leaves from each experimental plant and counted the number of leaves damaged by herbivores. To determine water table depths in the gardens, a water table well was installed at the center of each garden. The wells were built by attaching a commercial PVC well point (Campbell Manufacturing, LLC) to a section of PVC pipe of the same inner diameter (5cm). The well points had a closed bottom and fine slots on the walls to allow water moving in and out of the wells. The wells were installed 0.6 m below ground in wetland gardens and 1.5 m below ground in upland gardens. Water table depth was measured using a steel tape measure bi-weekly during the 2012 growing season.

Plant traits data collection

In August 2012, we collected two uppermost, fully expanded leaves from each experimental plant. One of the two leaves was used for measuring percentages of leaf nitrogen and carbon. These leaves were oven-dried at 60°C and analyzed using dry

combustion gas chromatography on a Costech Analytical ECS 4010 system (Costech Analytical Technologies Inc., Valencia, CA, USA). The other leaf was used to measure concentrations of CTs and PGs. These leaves were put into individual zip-lock bags filled with silicon gels. The bags were kept in a dark icebox and transferred to a lab where they were placed in a 4°C refrigerator until the leaves were completely dried, following the drying method in Julkunen-Tiitto et al. (1996). CTs and PGs were extracted from dried leaves following the protocol in Lindroth et al. (1993).

The total CTs concentration in leaves was measured using butanol acid assay (Porter et al. 1985) and standard purified from aspen leaves (*Populus tremuloides*). PGs were analyzed using high performance liquid chromatography (HPLC) on an Agilent 1200 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) following the method in Julkunen-Tiitto et al. (1996). The Salicaceae family contains approximately 20 different PGs; however, we were only able to obtain purified standards for five PGs, which were salicin, salicortin, HCH-salicortin, tremulacin, and tremuloidin. We identified these five PGs in the samples by comparing retention times of unknown peaks to the retention times of the standards, and identified the rest of the PGs in the samples by examining spectra of unknown peaks, as the spectra of PGs have a characteristic shape with a peak absorbance at 220nm. We recorded the number of PGs found in each leaf sample and calculated the total PG concentration of the sample as the total peak area at 220 nm of all PGs divided by the extracted dry leaf mass.

Data analysis

All analyses were conducted using the R program (R Core Team 2016).

To examine whether or not the real cage treatment effectively reduced herbivory, we performed a logistic regression of the percentage of damaged leaves, modeled as a binomial variable, on the cage treatment.

To examine the responses of species growth to the water table depth gradient and the effect of herbivory on species growth, we fitted 72 alternative linear mixed models to plant growth and selected the best models based on Akaike Information Criterion corrected for small sample size (AICc Sugiura 1978, Hurvich and Tsai 1991). The fixed effect formulas of these models include two parts. The first part models plant growth in real cages, and the second part models the reduction of growth caused by herbivory on plants in sham cages, i.e., the cage treatment effect.

We modeled plant growth in real cages using eight different fixed effects formulas based on alternative hypotheses about species tolerance to hydrologic stresses. Fixed effects included species, two functions of growing season maximum water table depth (hereafter referred to as water table depth), and interactions between species and the water tale depth effects. Across hydrologic gradients, the severity of drought and flooding stresses vary in opposite directions. Species could respond to only one of the two stresses, therefore the response of its growth would change monotonically across hydrologic gradients, which we modeled using a linear function of water table depth. Alternatively, a species could be sensitive to both types of water stresses, therefore the response of its

growth to the hydrologic gradient would be a hump-shaped curve, which we modeled using a quadratic function of water table depth.

We modeled cage treatment effect on growth using nine different fixed effect formulas that correspond to the following alternative hypotheses: (a) the cage treatment had no effect on plant growth; (b) the cage treatment effect was equal among species and constant across the hydrologic gradient; (c) the cage effect was different between species but constant across the hydrologic gradient; (d) the cage treatment effect was equal among species but varied across hydrologic gradients linearly; (e) the cage treatment effect was equal among species but varied across hydrologic gradients following a quadratic function; (f) the cage treatment effect was different among species and varied across hydrologic gradients linearly; (g) the cage treatment effect was different among species and varied across hydrologic gradients following a quadratic function; (h) the cage treatment effect was different among species and varied across hydrologic gradients linearly, and there was a species by water table depth interaction effect; (i) the cage treatment effect was different among species and varied across hydrologic gradients following a quadratic function, and there was a species by water table depth interaction effect.

Using the full combinations between the eight formulas for growth in real cages and the nine formulas for the cage treatment effect on growth, we constructed 72 models of plant growth. Besides the fixed effects, we also included a random site effect in all the models. If the best model(s) selected based on AICc included a three-way interaction

effect between species, water table depth, and the cage treatment, then it would support the hypothesis that the effect of herbivory varied across the water table depth gradient differentially between the species.

The effect of herbivory on plant growth depends on both the amount of herbivory and plant tolerance to herbivory damages. To examine whether the variation in the cage treatment effect on plant growth was caused by variation in the amount of herbivory between species and/or across the hydrologic gradient, we fitted eight generalized linear mixed models to herbivory on plants in sham cages and selected the best models based on AICc values. Herbivory was modeled as a binomial variable. The predictor formulas of the herbivory models were the same as the predictor formulas for the cage treatment effect in growth models that correspond to hypotheses (b) to (i).

To examine whether the variation in the amount of herbivory between species and/or across hydrologic gradients was caused by variations in leaf traits, we performed two analyses. First, we tested whether it was possible that variations in traits contributed to the variation in herbivory. If a trait was correlated with herbivory, and if it varied between species and/or across the hydrologic gradient, then it is possible that the variation in herbivory was partially caused by the variation in this trait. We first performed univariate regressions of herbivory on plants in sham cages on each of the traits alone to examine their correlations with herbivory. Based on existing literatures, we hypothesized that herbivory would increase with percent of leaf nitrogen, and decrease with percent of leaf carbon, CTs concentration, PGs concentration, and the number of

PGs. Then, we examined variations in traits between species and across the hydrologic gradient by fitting eight alternative models for each trait and selected the best model(s) based on AICc values. The predictor formulas of the traits models were the same as the fixed effects formulas of the herbivory models.

Second, we tested whether the variation in herbivory could be *solely* explained by variations in traits. If this was true, then a herbivory model including species, water table depth, and traits as predictors should have a worse fit (i.e. a higher AICc value) than herbivory models including only traits or only species and water table depth effect as predictors. Therefore, we compared three models of herbivory with different predictors. The first model is the previously selected best herbivory model, which we will refer to as the "herbivory-species/water table depth model" hereafter. The second model includes only traits as fixed effect and a random site effect, which we will refer to as the "herbivory-traits model" hereafter. Preliminary analyses showed that some traits were correlated with each other; therefore instead of including all the traits in the "herbivory-traits model", we used a step-wise model building strategy to built the "herbivory-traits model", adding one trait to the model at a time and starting from the trait showed the strongest correlation with herbivory. If adding a new trait did not reduce model AICc, then we would not include it to the model. The third model includes species, water table depth, and the traits included in the "herbivory-traits model", which we will refer to as the "herbivory-everything" model hereafter.

For all the above-mentioned analyses, we fitted the linear mixed effect models and generalized mixed effect models using the method of Maximum Likelihood implemented by the *lmer* and the *glmer* function in the *lme4* package. We computed model AICc values using the *AICc* function in the *AICcmodavg* package. If the difference in AICc values between two models was less than two, we considered them fit equally well to the data. If there were more than one best model, we computed 95% confidence intervals of averaged model parameters using the *modavg* function in the *AICcmodavg* package.

We tested the significance of phylogenetic signals in the five leaf traits and the concentrations of each individual PG that occurred in more than one species using the Blomberg's K statistic (Blomberg *et al.*, 2003). We used a published phylogeny constructed by five chloroplast barcode genes and a nuclear alcohol dehydrogenase gene (Savage & Cavender-Bares, 2012). We tested if the observed K values were significantly higher than the means of two null distributions: a random null distribution generated by randomly swapping the trait values across the tips of the phylogeny with 1000 replications and a Brownian null distribution generated by simulating trait evolution assuming a Brownian model using the sim.char function in the GEIGER package (Harmon *et al.*, 2008) with 1000 replications.

Considering the relative small sample size of this phylogenetic analysis (14 spp.), we also performed a jackknife analysis of phylogenetic signals in traits. Specifically, we removed one species at a time, re-tested phylogenetic signals in each trait 14 times using the same method mentioned above, and compared the results of the regular analysis

including all species against the means and standard deviations of the results of the jackknife analysis.

Results

Did the cage treatment effect on plant growth vary across hydrologic gradients differentially among species?

The proportion of damaged leaves was significantly higher in sham than real cages (0.26 vs. 0.16, $p<0.001$).

The AICc values of the growth models ranged from -3264.6 to -3179.5 (Table 2.1). Six models had equally best fit to the data (i.e. their differences in AICc values were less than two). None of these models included a three-way interaction between species, water table depth, and cage treatment. The results of model averaging indicated that plant growth was lower in sham cages than in real cages and increased with wetter water table depth (Figure 2.1, 2.2).

Was the variation in herbivory effect on plant growth caused by variations in the amount of herbivory damages and the leaf traits?

The AICc values of the herbivory models ranged from 1439.2 to 1618.7 (Table 2.2). The AICc value of the (only) best model is 13.5 units lower than that of the second best model. The fixed effects of the best model include species and a quadratic, concave

function of water table depth, without any interaction between species and water table depth (Figure 2.3).

The results of the univariate regressions indicated that herbivory decreased with the concentration and the number of PGs ($p<0.0001$), increased with the concentration of CTs ($p<0.0001$) and the percent of leaf nitrogen ($p=0.0001$), and showed no significant relationship with the percent of leaf carbon ($p=0.76$) (Figure 2.4). The best model of leaf percent carbon, CTs concentration, and PGs concentration included only a species effect (Appendix 8). Leaf percent nitrogen and the number of PGs had two equally best models (Appendix 8), one included only a species effect, and the other included both species and water table depth effects (but no interaction between the two). We averaged parameters between the two best models for these two traits and calculated 95% confidence intervals for the averaged parameter estimates. For both traits, only species, but not water table depth had a confidence interval that did not cover zero (Appendix 9).

The predictors of the "herbivory-traits model" included the number of PGs, CTs concentration, and the percent of leaf nitrogen. The AICc value of the "herbivory-everything model" (763.3) was lower than the AICc values of the "herbivory-traits model" (816.3) and the "herbivory-species/water table depth model" (801.7). These results indicate that the variation in herbivory could not be solely explained by variations in traits. We further tested whether the variation in herbivory between species could be solely explained by variations in traits. We fitted another herbivory model that had the same predictors as the "herbivory-traits model", but with an additional species effect.

AICc value of this model (789.2) was still lower than that of the "herbivory-traits model" (816.3), indicating the variation in herbivory between species could not be solely explained by variations in traits.

Did the leaf traits showed phylogenetic signals?

The observed K value of the number of PGs was significantly higher and marginally significantly higher than the mean of the random null K distribution and the mean of the Brownian null K distribution, respectively (K_{obs}= 1.79, K_{rand}=0.30, P<0.0001; K_{BM}=1.00, P=0.07). The observed K value (K_{obs}) of CTs concentration was marginally significantly higher than the mean of the random null K distribution (K_{obs}= 0.52, K_{rand}=0.31, P =0.07), but not significantly different from the mean of the Brownian null K distribution. Percentage of leaf nitrogen and carbon, as well as total PGs concentration did not show any significant phylogenetic signal against either null distribution (Figure 2.6, Appendix 10).

We also tested phylogenetic signals in the concentrations of eight individual PGs present in more than one species. Among the eight PGs, the observed K value of salicin concentration was significantly higher than the mean of the random null K distribution and the Brownian null K distribution (K_{obs}= 2.09, K_{rand}=0.34, P<0.0001; K_{BM}=1.00, P=0.05). The observed K values of concentrations of isosalicin and the PG6 were significantly and marginally significant higher than means of random null K distributions, respectively (K_{obs}= 1.45, K_{rand}=0.35, P=0.01; K_{obs}= 1.02, K_{rand}=0.39, P=0.05). None of

the other individual PG concentration showed a significant phylogenetic signal against either null K distribution (Figure 2.6, Appendix 11).

The results of the jackknife analysis were qualitatively the same as the analysis including all species and reported in Appendix 12.

Discussion

Insect herbivory did not affect beta diversity among the Salicaceae communities

The results of our study do not support the hypothesis that insect herbivory affected beta diversity among the Salicaceae communities. Although herbivory reduced plant growth, and varied between species and across hydrologic gradients, it did not show any species by water table depth interaction effect.

The quadratic, concave response of herbivory to hydrologic gradients is likely caused by factors besides the leaf traits examined in the study, as none of these traits showed significant variation across hydrologic gradients. In this study, we focused on traits associated with leaf chemistry. Some physical defense traits, such as leaf toughness and trichome density are known to be associated with drought tolerance. These traits might have changed along hydrologic gradients and contributed to the observed variation in herbivory. Besides plant traits, herbivore abundance could also change with hydrologic environments and cause variation in herbivory. For instance, at the wet end of the hydrologic gradients, insect herbivores that overwinter in soils might be excluded from these habitats due to waterlogging.

In contrast to the variation in herbivory between species and across hydrologic gradients, the best growth model included no interaction effect between cage treatment and species or water table depth, indicating that the cage treatment effect on growth was constant across species and hydrologic gradients. The difference between the herbivory and the growth models has two possible explanations. First, the cage treatment effect on growth depends on both the amount of herbivory and plant tolerance to herbivory damages. Tolerance to herbivory could vary between species and hydrologic gradients, cancelling out the impact of variation in herbivory on the cage treatment effect on growth. Second, the real cage treatment reduced but did not eliminate herbivory damages; therefore its effect on growth might not reflect the total effect of herbivory on growth. To differentiate between the two possibilities, we performed an *ad hoc* analysis comparing the same growth models as we did before, but replaced the cage treatment effect in the formulas of these models by the amount of herbivory damages. If tolerance to herbivory varied between species and/or across hydrologic gradients, the predictor formula of the best growth model should include interaction between herbivory and species and/or interaction between herbivory and water table depth. It turned out that none of such interaction effect was included in the best model (Appendix 13). Note this result does not invalid the conclusion that herbivory did not affect plant beta diversity among the Salicaceae communities, because neither herbivory nor plant tolerance to herbivory showed a species by water table depth interaction effect.

One unexpected result from our trait analyses is that herbivory damages increased with CTs concentration. This result should not be interpreted as herbivores preferring leaves with a higher concentration of condensed tannins. Instead, the positive correlation between herbivory and CTs concentration was driven by a trade-off between CTs and PGs concentrations. PGs and CTs are both synthesized from the Shikimic pathway (Boeckler et al., 2011; Barbehenn & Constabel, 2011). The trade-off is likely caused by competition for precursors or catalytic enzymes between the two groups of compounds. Two species in this study, *S. candida* and *S. eriocephala*, did not show any detectable amount of phenolic glycosides. When examined among individuals of these two species only, the positive correlation between herbivory damages and CTs concentration became no longer significant.

To date, a few studies examining the effect of insect herbivory on plant beta diversity (Fine et al. 2004, 2006; Brenes-Arguedas et al. 2009), including this one, have found mixed results. Although the number of studies is rather small, we suspect that this idiosyncrasy may be widespread, because multiple factors can influence the effect of herbivory on plant performance. These factors include plant resistance to herbivory, plant tolerance to herbivory, and herbivore abundance, all of which could change with abiotic environments. And the specific nature of the responses of these factors to abiotic environments could vary from system to system. For instance, while concentrations of plant secondary metabolites can change along resource availability gradients, the

direction and magnitude of such changes depend on the types of compounds (Koricheva et al. 1998).

Closely related Salicaceae species did not share similar secondary chemistry

The results of our study partially support the hypothesis that closely related Salicaceae species shared similar defense traits. While the number of PGs was significantly phylogenetically conserved, phylogenetic signals in concentrations of CTs and PGs were weak or insignificant. Furthermore, individual PGs showed different phylogenetic signals between each other, ranging from no signal to highly conserved.

The difference in phylogenetic signals between the total concentration of PGs and PG richness suggests that former was more evolutionary labile than latter. Theories on the evolution of plant defenses predict that the total investment of defenses depend on herbivory pressure (Feeny 1976) or resource availabilities (Coley et al. 1986). The low phylogenetic signals in PGs and CTs concentrations might reflect fluctuations in these environmental factors over evolutionary times. A different, but non-mutually exclusive explanation is that the liability itself could be adaptive. The fact that different PGs showed different phylogenetic signals indicates that the evolution of different compounds were not synchronized with each other, i.e., besides the total investment in chemical defenses, the secondary chemistry profile also changed through evolutionary times. Such a "moving target" might be more difficult for specialist herbivores to adapt to.

Traditionally, secondary chemistry was thought to be similar among closely related plant species. One of the most prominent hypothesis in the evolution of plant defense, the coevolution hypothesis proposed by Ehrlich and Raven (1964) predicts that closely related plant species should share similar chemical defenses. Recently, an increasing number of studies, including this one, show that close related plant species within the same families or genera could be dissimilar in their secondary chemistry (e.g., Bercerra 1997; Kursar et al. 2009; Rasmann & Agrawal 2011; Johnson et al. 2014; Cacho et al. 2015). Dissimilarity in defense chemistry may facilitate coexistence among closely relatives through density-dependent effects, particularly if they share similar requirements for resource availabilities and abiotic conditions. The Salicaceae species showed a significant phylogenetic signal in their distributions of along hydrologic gradients (Savage & Cavender-Bares 2012). The lack of phylogenetic signal in defense chemistry indicates that close relatives co-occurring in the same habitats tend to have different defense chemistry, which might provide a mechanism maintaining the alpha diversity of the Salicaceae species within local communities.

Conclusion

The results of our study did not support the hypothesis that insect herbivory promoted beta diversity among the Salicaceae communities across hydrologic gradients. While herbivory reduced plant growth, and was different between species and showed a quadratic, concave response across hydrologic gradients, there was no species by water

table depth interaction effect on herbivory. Variation in herbivory between species, but not across hydrologic gradients was partially attributed to variations in leaf traits related to defense chemistry and nutrient quality.

On the other hand, herbivory might have contributed to the maintenance of alpha diversity within the Salicaceae communities. Although the number of PGs was highly phylogenetically conserved, phylogenetic signals in concentrations of PGs and CTs were insignificant or weak. Given that closely related Salicaceae species tended to share similar habitats along hydrologic gradients, differences in defense chemistry among the co-occurring close relatives might promote their coexistence within local communities through density-dependent effects.

Table 2.1 AICc values of the growth models. We fitted 72 growth models and selected the best models based on AICc values. In each cell of the table below is the AICc value of a candidate model. The fixed effect formulas of the models have two parts. The first part is for growth in real cages, which is shown in the column names of the table. The second part is for the cage treatment effect, which is shown in the row names of the table. The best models are highlighted in grey background. "c" represents cage treatment, "wtd" represents water table depth, and "sp" represents species.

	Intercept	wtd	wtd+wtd^2	sp	sp+wtd	sp+wtd+wtd^2	sp*wtd	sp*(wtd+wtd^2)
No cage effect	-3257.6	-3259.3	-3259.4	-3259.5	-3262.0	-3261.5	-3250.4	-3250.2
c	-3260.3	-3261.9	-3261.9	-3262.2	-3264.6	-3264.1	-3252.7	-3250.9
c+c:sp	-3240.6	-3242.0	-3241.8	-3244.4	-3246.8	-3246.4	-3233.7	-3226.0
c+c:wtd	-3262.0	-3260.7	-3260.7	-3264.8	-3263.5	-3262.9	-3251.9	-3248.7
c+c:(wtd+wtd^2)	-3241.9	-3240.5	-3240.4	-3245.2	-3245.1	-3244.7	-3232.4	-3223.6
c+c:sp+c:wtd	-3224.9	-3223.3	-3222.9	-3227.8	-3226.6	-3226.0	-3219.4	-3204.2
c+c:sp+c:(wtd+wtd^2)	-3260.6	-3259.5	-3258.7	-3263.1	-3262.0	-3260.8	-3250.6	-3246.3
c+c:(sp*wtd)	-3240.2	-3239.0	-3238.2	-3244.5	-3243.7	-3242.4	-3231.6	-3220.9
c+c:(sp*(wtd+wtd^2))	-3202.3	-3200.7	-3199.8	-3203.8	-3202.6	-3201.2	-3194.4	-3179.5

Table 2.2 AICc values of the herbivory models. We fitted eight alternative models for herbivory damages on plants in sham cages and selected the best model based on AICc values. The best model, highlighted in grey background, includes a species effect and a quadratic function of water table depth. "wtd" is the abbreviation for water table depth.

Fixed effect formulas	**AICc**
wtd+wtd^2+species	1439.2
wtd+wtd^2+species+species:(wtd+wtd^2)	1452.7
wtd+species	1459.2
wtd+species+species:wtd	1461.4
species	1465.9
wtd+wtd^2	1590.4
wtd	1609.9
intercept	1618.7

Figure 2.1 95% confidence intervals of parameter estimates averaged across the six best growth models (see Table 2.1). Solid dots are the averaged parameter estimates; solid horizontal lines represent 95% confidence intervals around the means. Dash vertical line represents zero. "wtd" is the abbreviation for water table depth.

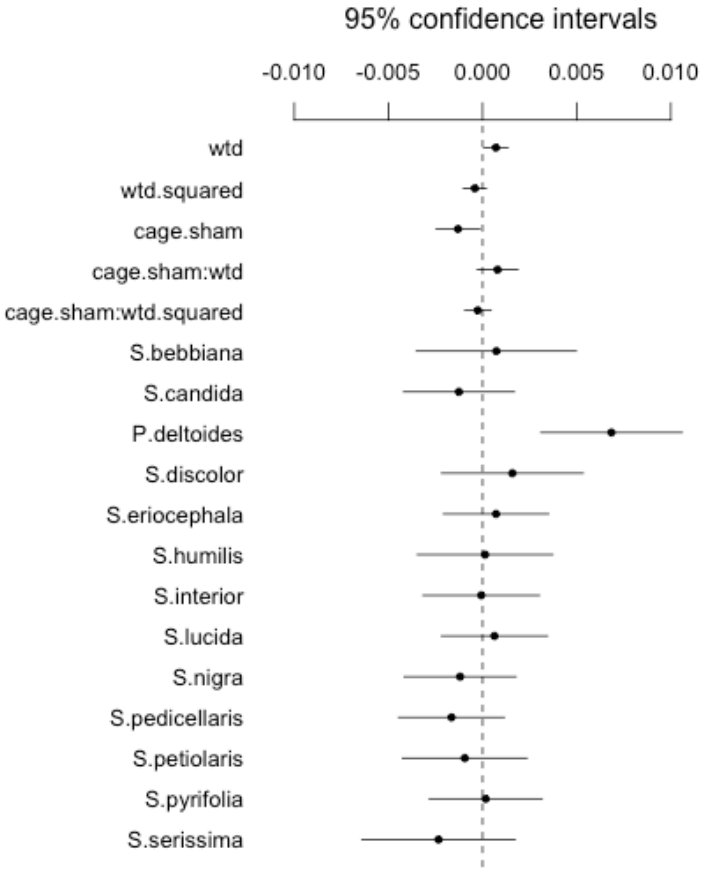


Figure 2.2 Observed and predicted species growth rate across water table depth gradients. Orange circles and dash curves are the observed and predicted growth of species in real cages; blue circles and solid curves are the observed and predicted growth of species in sham cages. Species are indicated with the first letters of their species names. The predictions were made based on parameter estimates averaged across sixe best growth models (Figure 2.1), without taking into account of the site random effect.

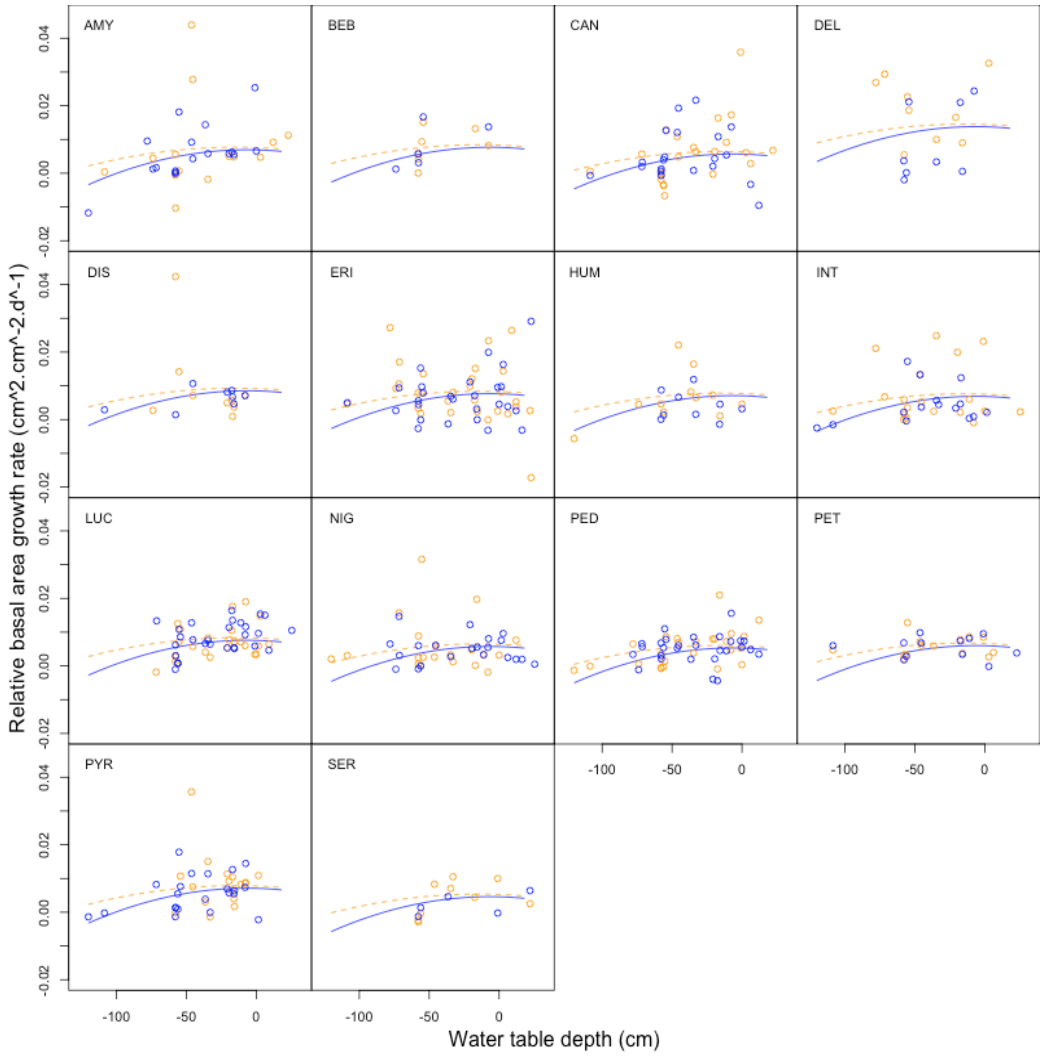


Figure 2.3 Variation in herbivory between species and across the water table depth gradient. (a) Species means proportion of damaged leaves. Species are indicated with the first three letters of their species names. (b) Residuals of herbivory after regressing against a fixed species effect and a random side effect, plotted against water table depth. The solid curve represents the least square regression curve of the herbivory residual on water table depth.

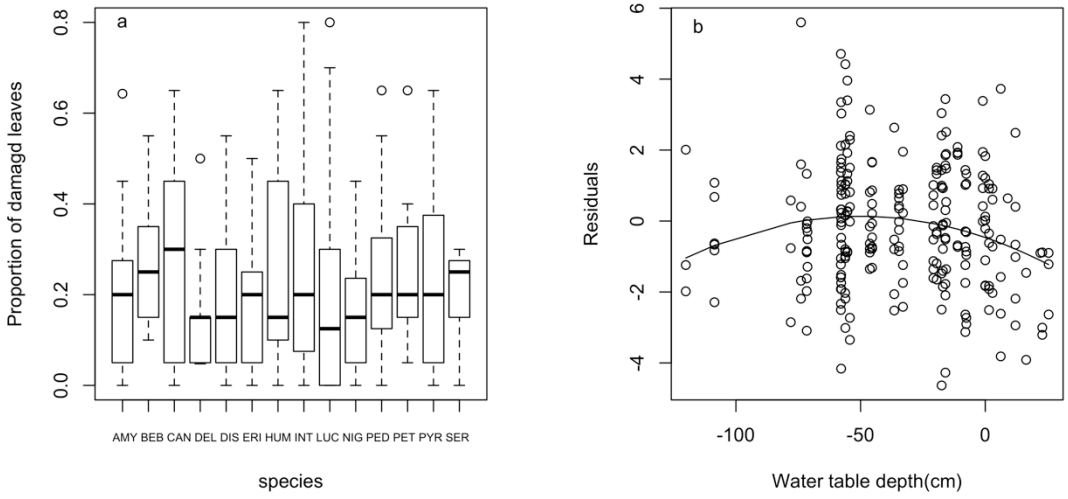


Figure 2.4 Logistic regressions of herbivory on (a) leaf percent nitrogen, (b) condensed tannins concentration, (c) phenolic glycosides concentration, and (d) the number of phenolic glycosides. Solid curves are logistic regression curves.

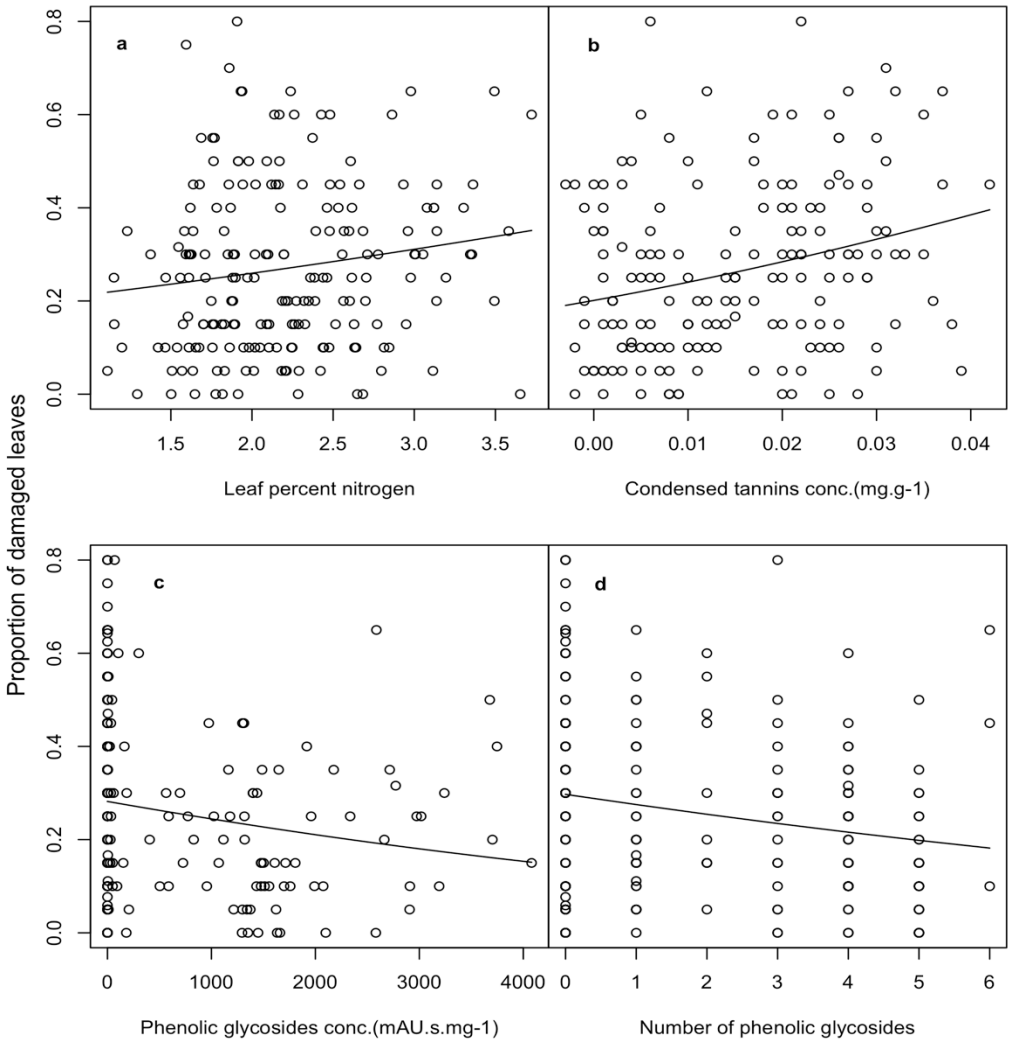


Figure 2.5 Variations in traits between species and across hydrologic gradient. Species mean leaf percent nitrogen (a), percent carbon (c), condensed tannins concentration (e), phenolic glycosides concentration (g), and the number of phenolic glycosides (i). Residuals of leaf percent nitrogen (b), percent carbon (d), condensed tannins concentration (f), phenolic glycosides concentration (h), and the number of phenolic glycosides (j) across the water table depth gradient, after regressing these traits against species.

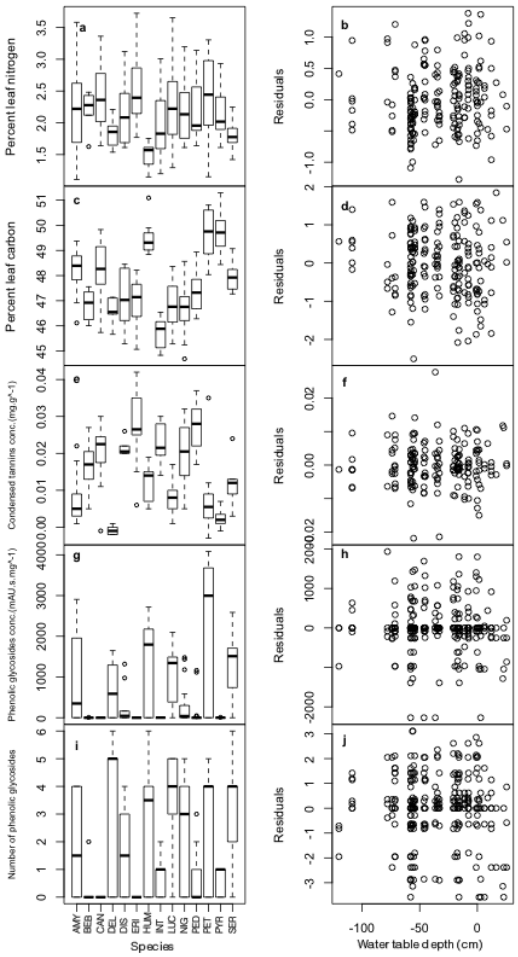
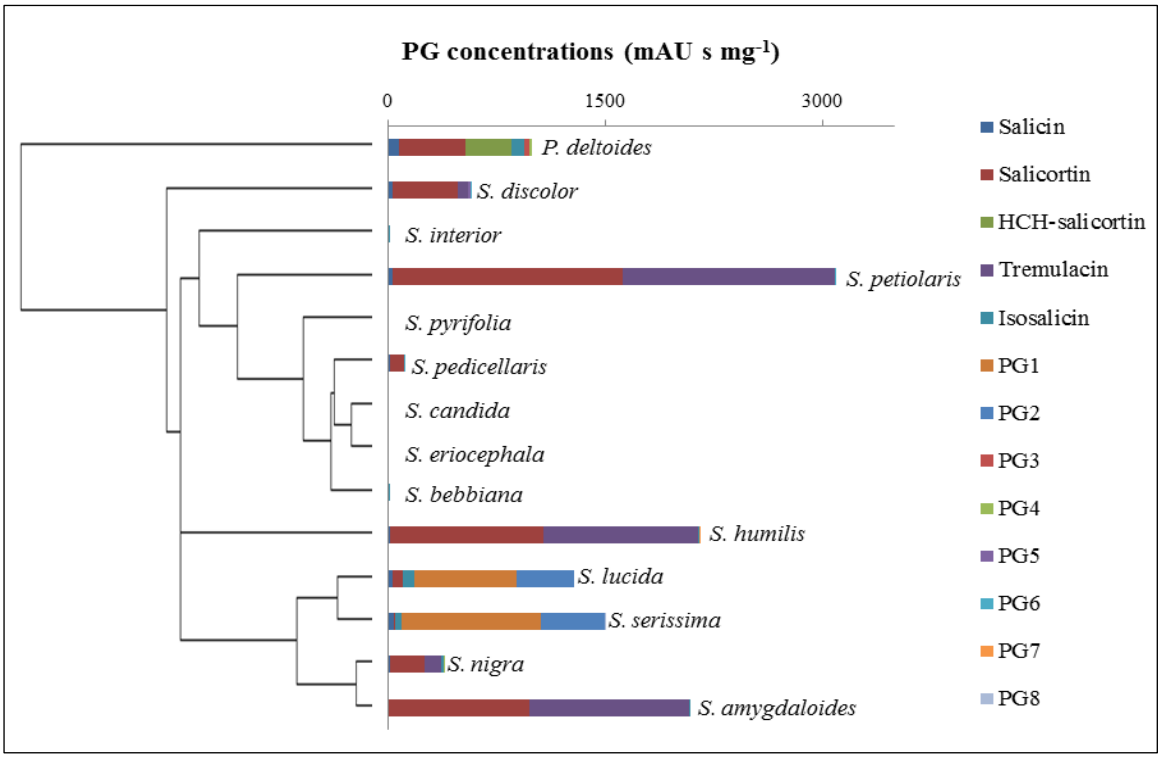


Figure 2.6 Distributions of phenolic glycosides concentrations across phylogeny.



Growth and physiological responses to flooding in seven willow (genus *Salix*) species co-occurring along hydrologic gradients

Summary

Terrestrial plants often show differential distributions along hydrologic gradients based on their tolerances to water stresses. In this study, we performed a greenhouse experiment to examine responses of growth and physiological traits in seven co-occurring willow species to a six-week flooding treatment followed by another six-week recovery period. The flooding treatment increased plant growth and carbon assimilation by improving plant water status. Plants received the flooding treatment showed higher stomatal conductance and predawn leaf water potential than plants received the control treatment. Species distributed in wetter habitats had higher stem growth rates in the flooding treatment; and species distributed in habitats with greater water table depth fluctuations showed greater variations in growth between the flooding and the recovery period. Together, the results of our experiment suggest differential tolerances to flooding and water table depth fluctuation may contribute to habitat segregation among the willow species.

Key words

Flooding tolerance, hydrologic gradients, habitat segregation, willow (*Salix*).

Introduction

Water is an organizational factor for terrestrial plant communities. Along hydrologic gradients, both drought and flooding could act as drivers of environmental filtering, influencing species distributions and community assembly. Human activities have been altering hydrologic regimes around the globe (Jackson et al. 2001; Huntington 2006; Barnett et al. 2008). Predicting plant community responses to such alterations requires a mechanistic understanding of the ecological processes influencing species distributions along hydrologic gradients and of the functional traits underlying species tolerances to water stresses.

While numerous studies have examined the role of drought in plant community assembly along hydrologic gradients and the functional traits underlying drought tolerance, relatively fewer attentions have been paid to the wet ends of hydrologic gradients. Flooding can reduce the growth of or even cause mortality in sensitive plants. It primarily cause stress in plants by reducing oxygen availability in soils and inhibiting roots respiration, which interrupts the uptake of water and mineral nutrients (Armstrong 1979; Vartapetian & Jackson 1997; Voesenek *et al.* 2004). The negative impacts of flooding on root functioning can in turn, affect the functioning of aboveground tissues. A commonly observed plant response to flooding is reduction in stomatal conductance and photosynthesis, which is thought to be associated with the reduction in roots hydraulic conductance (Crane & Davies 1989; Kozlowski T. 1997). Besides negatively affecting plant water status, plant can also cause nutrient deficiency and chlorosis, which further

reduces photosynthesis and growth (Davies & Flore 1986; Pezeshki 1994; Yordanova *et al.* 2004).

Although most terrestrial plants are sensitive to flooding, some species thrive in wetlands. These plants have evolved various adaptions to cope with the flooding stress (Colmer & Voesenek 2009; Catford & Jansson 2014). For instance, when exposed to flooding, some species produce adventitious roots to replace dead roots killed by anoxia. Adventitious roots have higher porosities than normal roots, which are thought to facilitate gas transportation (Kozlowski 1997; but see Armstrong *et al.* 1994; Jackson & Attwood 1997). Lenticel is another trait that is known to be associated with flooding tolerance. These openings on stem epidermis, particularly those located above water tables, are entries of oxygen to waterlogged tissues (Armstrong 1968).

Flooding tolerance traits, such as adventitious roots and lenticels, improve aeration in flooding tolerant plants and allow them to maintain root functioning. In fact, some species adapted to wetlands are found to perform better under flooded than non-flooded conditions (Amlin & Rood 2001; McKevlin *et al.* 1995). One reason for these species to benefit from flooding is improved water status. For instance, a glasshouse experiment showed that *Paspalum dilatatum*, a wetland weed, had higher leaf water potential, stomatal conductance, and transpiration in flooding than control treatment when there was a high water evaporative demand (Striker *et al.* 2006, 2007). Furthermore, flooding-tolerant plants can also absorb more mineral nutrients under flooded conditions (Dickson *et al.* 1972; Hosner & Leaf 1962).

Besides reducing oxygen availability, another way that flooding can cause stress in plants is the fluctuation of water table depth itself. When water table drops and oxygen concentration increases in previously waterlogged tissues, plant cells may experience post-anoxia injuries caused by Radical Oxygen Species (ROS). Under normal conditions, ROS are constantly detoxified by protective enzymes or non-enzymatic systems, which can be deactivated under anoxia conditions. Flooding tolerant species are able to avoid post-anoxia injuries either by rapidly ramping up their ROS protective systems after oxygen concentration increases (Ushimura et al. 1992) or keeping them activated under anoxia condition (Monk 1987; Weber & Braendle 1996). Furthermore, traits that are beneficial for surviving flooding stress can become disadvantageous after floods recede. For instance, while adventitious roots help to maintain water and nutrients uptake during flooding periods, they are often concentrated right beneath water tables and can be dried out when water tables drop. In such cases, species that did not preserve the viability of their deeper roots during flooding periods may risk desiccation (Crawford 1996).

Species in the Salicaceae family provides a very suitable system for examining flooding tolerance studies. Even though the Salicaceae species are generally considered flooding tolerant compared to other woody plants, there is variation in flooding tolerance among the species (e.g., Amlin & Rood 2001). At our study site, Cedar Creek Ecosystem Science Reserve (CCESR), naturally occurred Salicaceae species distributed in habitats with differential mean water table depths and within-growing season water table depth fluctuation (Savage & Cavender-Bares, 2012). It is remains to be tested whether the

Salicaceae species are different in their flooding tolerance and whether such differentiation is predictive of the differences in their distributions along hydrologic gradients.

In this study, we compared flooding tolerance among seven willow (genus *Salix*) species occurring at CCESR using a glasshouse experiment. Cuttings of the species were exposed to a six-week period of flooding treatment, followed by a recovery period of the same length. Growth, biomass allocation, and multiple physiological traits were measured to access species' responses to the flooding treatment. We also measured two traits that are known to be associated with flooding tolerance, namely lenticel density and the production of adventitious roots. We aimed to address two questions. First, were species responses in growth and physiological traits to the flooding treatment correlated with their distributions along hydrologic gradients? Specifically, we hypothesized that species with higher mean water table depths in their natural habitats should have better performances in the flooding than the control treatment, i.e. they should have higher growth rate, photosynthesis rate, stomatal conductance, leaf water potential, chlorophyll content, and lower non-photochemical quenching. We also hypothesized that differences in growth and physiological traits between the flooding and the recovery period should be smaller in species with greater water table depth fluctuations in their natural habitats. Second, were the species showed more positive growth responses to the flooding treatment had higher lenticel densities and produced more adventitious roots?

Materials and methods

Greenhouse experiment design

In fall 2012 and spring 2013, we collected cuttings of seven willow species from their natural populations at CCESR. Savage & Cavender-Bares (2012) found 14 Salicaceae species co-occur at CCESR and show differential distributions along hydrologic gradients. Briefly, they established 50 random plots at CCESR and measured the basal area abundance of Salicaceae species and monitored water table depth within the plots for two years. From the abundance and the water table depth data, they calculated two metrics indicating species' hydrologic niches: the average growing season maximum and minimum water table depth of the species weighted by their abundances in the plots (hereafter as WT_{wet}, WT_{dry}). We selected seven out of the 14 species based on WT_{wet} and the differences between WT_{wet} and WT_{dry}; the latter reflects the magnitude of water table depth fluctuation in the species' natural habitats (Table 3.1).

The cuttings were brought back to the Greenhouse Facility at the University of Minnesota, St. Paul, where they were soaked in tap water for a week and then planted in small pots filled with moist sands to root. Once the plants developed healthy roots, they were transferred to larger pots filled with a mixture of sand, potting soil, and sphagnum moss. The plants were kept in a greenhouse room with ambient temperature and photoperiod, except that the plants collected in 2012 fall overwintered at 2-Celsius degree.

The experiment was conducted at the Greenhouse Facility of University of Minnesota, from July to October 2013. Six mental tanks were set up in a greenhouse

room. Three tanks received the flooding treatment and the other three tanks were controls. The flooding treatment tanks were filled with tap water during the first six weeks of the experiment, while the control tanks had no water. After six weeks, the water in the flooding treatment tanks was drained and the experiment continued for another six weeks. Nine to 37 plants were selected from each species and divided equally among the tanks. We were not able to include equal number of plants from each species due to the differential survival rates among the species. The experimental plants were kept in individual pots. The pots were put on plastic racks, so that tops of the pots were at the same height as edges of the tanks. Positions of pots within tanks were randomized. The soils inside the pots were kept at five centimeters below the tops of the pots. During the flooding period, water filled the flooding treatment tanks, so that soils in the pots were saturated and there was five centimeters high standing water in the pots, partially submerging stems of the plants.

Growth and traits data collection

We measured basal diameter, stem height, leaf number, and average leaf length of each plant four times during the experiment: right before and at the fourth week during the flooding period, and at the third and the sixth week during the recovery period. We calculated relative growth rates of basal area, height, stem volume, leaf number, and leaf area for each plant during the flooding and the recovery period separately. Stem volume was calculated based on basal diameter and height, assuming stems had cylinder shapes.

Leaf area was calculated based on the total leaf number and the average length of 20 randomly sampled leaves, assuming leaves had circular shapes. All the relative growth rates were calculated assuming exponential growth models.

We measured gas exchange, chlorophyll fluorescence, chlorophyll content, and predawn leaf water potential four times during the experiment: twice during the flooding period, and twice during the recovery period. At each time, two upper most, fully expanded leaves were selected from each plant. One leaf was sampled for measuring water potential; the rest of the measurements were taken on the other leaf. We measured gas-exchange using a portable photosynthetic analyzer (LI-6400XT, Licor Inc., Nebraska, USA). For data analysis, we calculated area-corrected net maximum assimilation rate and stomatal conductance. Chlorophyll fluorescence was measured using a portable photosynthetic yield analyzer (Mini-Pam, Heinz Walz GmbH, Effeltrich, Germany). To measure dark-adapted minimum and maximum chlorophyll fluorescence (F_0, and F_m), a dark adaption leaf clip (Hansatech Instruments Ltd., Pentney, United Kingdom) was attached to the selected leaf during evenings. In the following morning, the minimum and maximum dark-adapted fluorescence were measured at 9-10 am. Then the shutter of the leaf clip were left open for one to two hours to allow the leaf to adapt to light before the light-adapted minimum and maximum chlorophyll fluorescence (F_s, and $F_{m'}$) were measured on the same spots on leaves. For data analysis, we calculated maximum photochemical efficiency [$F_v/F_m = (F_m-F_0)/F_m$], yield of photosynthesis [$\Delta F/ F_m' = (F_m'-F_s)/F_m'$], and non-photochemical quenching [$NPQ = (F_m'-F_m)/F_m'$]. Chlorophyll content

was measured using a portable polyphenol and chlorophyll meter (Force-A LLC, Orsay, France). Three measurements were taken at different spots on each leaf and averaged for analysis. Predawn leaf water potential was measured using a Scholander plant water status console (Scholander PWSC 3000, ICT international, Armidale, Australia). Leaves were collected prior to sunrise, wrapped in moist paper towel, and kept in sealed zip-lock bags in an icebox. Leaf petioles were trimmed with a razor blade right before the measurements were taken.

We measured lenticel densities on young branches and main stems three times during the experiment: before the flooding period, during the flooding period, and during the recovery period. Each time, we counted the number of lenticels on three internodes from the main stem and on a young branch of the each plant. The length and the diameter of each internode were measured to estimate the surface area of the internode, assuming it had a cylindrical shape. To calculate lenticel densities, we first calculated lenticel density of each internode as the number of lenticels per unit of surface area then averaged across three internodes.

We counted the number of adventitious roots in the standing water during the fourth week of the flooding period. Only the roots directly branched out from the main stem was counted. The plants with more than 20 roots were recorded as having “abundant” adventitious roots. For data analysis, we ranked the species by comparing the proportion of individuals that were classified as having “abundant” adventitious roots in each species.

For the species with no individuals having more than 20 adventitious roots, we ranked them by the average number of advantageous roots.

By the end of flooding period, we harvested six plants per treatment from each of the four species: *S. interior*, *S. eriocephala*, *S. petiolaris*, and *S. lucida* to examine their biomass allocation. These species were chosen because they had contrasting distributions across the water table depth gradients and large sample sizes. The plants were divided into shoots and roots; the latter was further divided by soil depth (0-10cm, 10-20cm, 20-25cm, adventitious roots if any), dried and weighed separately.

Data analysis

All analyses were performed using the R program (R Core Team 2016).

To examine species' responses to the flooding treatment, we fitted a series of mixed models to relative growth rates, physiological traits, and lenticel densities using the *lmer* function in the lme4 package, and compared these models based on Alkaike Information Criterion corrected for small sample size (AICc) using the *AICc* function in the *AICcmodavg* package. We fitted six models to each response variable. These models all included three fixed effects: species, time, and the interaction between the two, as well as two random effect: tank, and individual plant. Besides these effect, the models could a) had no other effect; b) had a treatment effect; c) had a treatment by species interaction effect; d) had a treatment by time interaction effect; e) had both the treatment by species and the treatment by time interaction effects; f) had a three way interaction effect

between species, treatment, and time. We then calculated 95% confidence intervals of the treatment effect terms in the best models based on likelihood profile using the *confint* function in the lme4 package. If the difference in AICc values between two models was less than two, we considered them fitted the data equally well. If there were more than one best model, we averaged parameter estimates across all the best models and calculated 95% confidence intervals of the averaged estimates using the *modavg* function in the *AICcmodavg* package.

To examine the effect of the flooding treatment on species biomass allocation, we performed a two-way ANOVA to test the effect of species, treatment, and the interaction between the two on total biomass and shoots to roots ratio in the four species harvested at the end of the flooding period. We also compared the proportion of adventitious roots in total biomass among species using a one-way ANOVA.

To examine correlations between species' growth responses to the flooding treatment and their natural distributions along hydrologic gradients, we performed two sets of regression analyses. First, we regressed log response ratios of species mean relative growth rates to the flooding treatment on WT_{wet}. Second, we regressed variations in species mean relative growth rates the flooding treatment between the flooding and the recovery period [=|RGR in the flooding period - RGR in the recovery period|/ (RGR in the flooding period + RGR in the recovery period)] on the difference between WT_{wet} and WT_{dry} (hereafter as DeltaWT).

Finally, to examine whether or not lenticel densities and adventitious roots were associated with flooding tolerance in the *Salix* species, we performed regressions of lenticel densities and the rank of adventitious roots abundance on WT_{wet} and on log response ratios of growth rates between the flooding treatment and the control treatment.

Results

Relative growth rates of basal area, stem volume, and height were higher in the flooding than the control treatment. Furthermore, such treatment effects were greater during the flooding than the recovery period (Table 3.2, Figure 3.1, Appendix 14-15). The best model of relative stem volume growth rate included a treatment, and a treatment by time interaction effect. The 95% confidence intervals of both effects did not overlap with zero. Relative growth rate of basal area and height had two best models: the same model as that of relative stem volume growth rate, and a model without any treatment effect. The confidence interval of the treatment effect, but not the treatment by time interaction of the best relative basal area growth models did not include zero. The confidence interval of the treatment by time interaction effect, but not the treatment effect of the best relative height growth models did not include zero. The best model of relative leaf number and leaf area growth rates did not include the treatment effect.

Maximum photosynthetic rate, predawn water potential, and non-photochemical quenching (NPQ) had two best models: one with and one without the treatment effect. Averaged parameter estimate of the treatment effect across the best models indicated

flooding increased photosynthetic rate and predawn water potential, and decreased non-photochemical quenching, but confidence intervals of averaged parameter estimates all included zero (Table 3.2, Figure 3.2a-c, Appendix 14-15).

Stomatal conductance had two best models: one without treatment effect, the other had a treatment and a treatment by time interaction effect (Table 3.2, Appendix 14). Averaged parameter estimates indicated that the flooding treatment increased stomatal conductance, and this effect was lower during the recovery than the flooding period (Appendix 15, Figure 3.2d). However, the confidence intervals of all these parameter estimates overlapped with zero (Appendix 15).

Chlorophyll content had two best models: one with a treatment by species interaction, the other with a treatment by species interaction and a treatment by time interaction effect (Table 3.2, Appendix 15). Averaged parameter estimates indicated that the flooding treatment decreased chlorophyll content. Furthermore, the treatment effect was different between species, and changed between times (Figure 3.2e). Only the confidence intervals of the treatment by species interaction effect did not include zero (Appendix 15).

The best models of maximum photosynthetic efficiency (F_v/F_m) and yield of photosynthesis in light ($\Delta F/ F_m'$) did not include the treatment effect (Appendix 14).

Lenticel density on young branches had two best models: one without any treatment effect, the other included a treatment, and a treatment by time interaction effect (Appendix 14). Averaged parameter estimates indicated that the flooding treatment

increased lenticel density on young branches and this effect was greater during the flooding than the recovery period (Figure 3.2f). Confidence intervals of the treatment by time interaction, but no the treatment effect, did not overlap with zero. The best model of lenticel density on main stems did not include any treatment effect (Appendix 15).

The total biomass of the four harvested species was significantly higher in the flooding than the control treatment, but there was no significant interaction between species and treatment (Table 3.3, Figure 3.3a). Shoots to roots ratio was significantly different among species, higher in the flooding than the control treatment, and there was a significant interaction effect between species and treatment (Table 3.3, Figure 3.3b). The proportion of adventitious roots was significantly different among species (F=3.19, P=0.05, Figure 3.3c).

The variation in species mean stem height relative growth in the flooding treatment between the flooding and the recovery period showed a marginally significant, positive correlation with DeltaWT (R^2=0.50, P=0.07, Figure 3.4a). None of the other growth rate responses showed any significant correlation with WT_{wet} or DeltaWT. There was also no significant correlation between flooding tolerance traits (lenticel densities and rank of adventitious roots abundance) and WT_{wet} or log response ratios of growth rates to the flooding treatment.

Discussion

Flooding improved performances of the willow plants by improving their water status

A six-week long flooding treatment did not seem to stress any of the species examined in this study. Instead, the flooding treatment increased stem growth rates and total biomass of the plants. Besides growth, the flooding treatment also had a positive effect on multiple physiological traits. The flooding treatment tended to increase predawn leaf water potential, stomatal conductance, carbon assimilation rate, shoots to roots ratio, and decrease non-photochemical quenching. These evidences suggest that the flooding treatment might have enhanced plant growth by improving their water status. Better water status might be particularly advantageous during warm summer days, when plants could be forced to reduce stomatal conductance and photosynthesis to avoid excessive loss of water.

The positive effects of the flooding treatment on plant stem growth decreased during the recovery period, which indicates that the *Salix* plants were able to adjust to changes in their hydrologic environments within a relatively short period of time. We also observed a trend of similar changes in stomatal conductance, which further support the hypothesis that the positive effect of the flooding treatment on growth was underlined by an improved water status.

In contrast to the effects of the flooding treatment on traits associated with water status, the effect of flooding treatment on a nutrient-related trait, chlorophyll content varied different between species. Interestingly, the flooding treatment had opposite effects on the two upland species: it significantly increased chlorophyll content in *S. interior* and decreased chlorophyll content in *S. humilis*. This result suggests that the

effect of flooding treatment on leaf nutrients was not correlated with species' distributions along hydrologic gradients. Alternatively, chlorophyll content might not be representative of total nitrogen pools in leaves. Besides chlorophyll, a considerable proportion of leaf nitrogen is invested in RuBisCo, which might show a different response to flooding from chlorophyll.

Species distributed in wetter habitats had higher growth rates in the flooding treatment

We did not found evidences support the hypothesis that the flooding treatment had more positive effects on the performance of species with wetter hydrologic niches. There was no species by treatment interaction effect on growth rates and the majority of physiological traits. Furthermore, none of the log response ratios of growth rates showed a significant correlation with species' hydrologic niches. Our hypothesis was based on the assumption of environmental filtering, i.e. species should be most abundant in the habitats where they had best performances along the hydrologic gradients. An alternative hypothesis is that species should be most abundant in the habitats where they had better performances than other species, i.e. species distributions was driven by competition rather than environmental filtering. In an *ad hoc* analysis, we found species mean relative basal area growth rates in the flooding treatment during the flooding period was positively correlated with species natural distributions along hydrologic gradients ($r=0.77$, $P=0.04$, Figure 3.4b) and water table depth optima predicted based on the field experiment ($r=0.75$, $P=0.05$), supporting the alternative hypothesis.

In a previous chapter, a field transplanting experiment provided evidences of environmental filtering in 14 Salicaceae species co-occurred at CCESR. While the previous results did not rule out the possibility that competition might influence species distributions along hydrologic niches, it does raise the question why we did not find evidences of environmental filtering in this greenhouse experiment. One possible explanation is that compared to this greenhouse study, the field experiment included a wider range of hydrologic conditions so that the experimental plants were exposed to more severe stresses. Particularly, a major contributor to the differences in fitness responses between species in the field experiment was the high floods occurred in early springs. Such floods completely submerged experimental plants in some of the wetland gardens and might have severely impacted photosynthesis. The flooding treatment in this greenhouse study only partially submerged the experimental plants, leaving most of their photosynthetic tissues in the air.

Our second hypothesis, i.e. species distributed in habitats with a greater water table depth fluctuation should show less variation in their growth (in the flooding treatment) between the flooding and the recovery period was also not supported by the results. There was a marginally significant, positive correlation between the variation in species' stem height growth rates and the magnitude of water table depth fluctuation in their natural habitats. This result is the opposite of our hypothesis. It suggests that the species distributed in more fluctuating hydrologic environments tended to be more plastic than species distributed in habitats with more constant water tables.

Lenticel density, but not adventitious roots abundance, was correlated with species growth rates in the flooding treatment

The flooding treatment tended to increase lenticel density on young branches during the flooding period, and this positive effect was reduced during the recovery period. These results suggested that a higher lenticel density might be involved in flooding tolerance in the *Salix* plants, possibly by facilitating aeration of waterlogged tissues.

We did not find any significant correlation between species mean lenticel densities and any of the log response ratios of growth rates. However, lenticel density on young branches was marginally significantly correlated with species mean basal area growth rates in the flooding treatment ($r=-0.71$, $P=0.08$). There was also a negative, insignificant correlation between lenticel density on young branches and hydrologic niches ($r=-0.43$, $P=0.33$). These results are in with the results of a previous chapter, in which lenticel density measured from the plants in the field experiment was also negatively correlated with species hydrologic niches.

Several lines of evidences suggest that adventitious roots production was not correlated with flooding tolerance among the *Salix* species examined in this study. First, the rank of adventitious roots abundance was not correlated with species' hydrologic niche, or any of the log response ratios of growth rates to the flooding treatment. Furthermore, among the four harvested species, the one with the lowest biomass allocation to adventitious roots, *S. pedicellaris*, is a wetland specialist mostly distributed in permanently saturated bogs. Adventitious roots have long been considered playing a

critical role in flooding tolerance. They are thought to replace to dead roots killed by anoxia and therefore necessary for maintaining root functioning under waterlogged conditions. However, some studies (e.g., Armstrong *et al.* 1994; Jackson & Attwood 1997), including ours, fail to find a correlation between adventitious roots production and flooding tolerance. These studies imply that adventitious roots production is not the only trait influencing flooding tolerance. There may be alternative tolerance strategies to deal with different types of flooding stress. Adventitious roots can only occur when plants are exposed to temporary flooding. They might be less advantageous for species that are adapted to permanently saturated soils, such as *S. pedicellaris*. On the other hand, species with higher ranks of adventitious roots abundance had higher predawn leaf water potential (R^2=0.94, P=0.0003) and stomatal conductance (R^2=0.72, P=0.02) among the seven species included in this study. It also increased with root biomass among the four harvested species. These correlations suggest that species' ability to produce adventitious roots in response to flooding was associated with their roots allocation and growth, which in turn, might have improved their water status.

Conclusion

Our experiment showed that a six-week flooding treatment improved species growth rates, carbon assimilation rates, and shoots to roots ratio by improving plant water status. The flooding treatment increased lenticel density on young branches; this positive effect disappeared during the recovery period. These results suggest lenticels on young

branches might facilitate aeration and improve flooding tolerance in the *Salix* species. On the other hand, the abundance of adventitious roots, a trait that is thought to be involved in flooding tolerance, was not correlated with species' growth responses to the flooding treatment.

Contrary to our hypothesis, species mean basal area growth rates in the flooding treatment, not the difference in species growth rates between the flooding and the control treatment, was significantly correlated with species' natural distributions along hydrologic gradients. Furthermore, the variation in stem height growth rates in the flooding treatment between the flooding and the recovery period was positively, instead of negatively correlated with the growing season water table depth fluctuation in species' natural habitats. Together, these results suggest differential responses to flooding and water table fluctuation might have contributed to habitat segregation among the willow species along hydrologic gradients.

Table 3.1. Hydrologic niches of the Salicaceae species. Species included in this study was highlighted in bold font and shade. These species were chosen to cover to full range of the hydrologic niches of 14 naturally occurred species at CCESSR. WT_{wet} and WT_{dry} are the mean growing season maximum and minimum water table depth weighted by the basal area abundance of natural populations, respectively (Savage and Cavender-Bares 2012).

Species	Abbreviation	WT_{wet} (cm)	WT_{dry} (cm)
S. interior	**INT**	**-102**	**-110**
S. humilis	**HUM**	**-83**	**-117**
P. deltoides	DEL	-77	-86
S. amygdaloides	AMY	-19	-40
S. eriocephala	**ERI**	**-18**	**-62**
S. petiolaris	**PET**	**-15**	**-43**
S. bebbiana	BEB	-18	-46
S. discolor	DIS	-3	-50
S. nigra	NIG	-1	-24
S. candida	CAN	1	-30
S. pyrifolia	**PYR**	**1**	**-4**
S. serissima	SER	2	-20
S. pedicellaris	**PED**	**4**	**-14**
S. lucida	**LUC**	**7**	**0**

Table 3.2 Treatment effects in the best models of growth rates, physiological traits, and lenticel densities. We compared six models of each of the response variables. The candidate models differed from each other by whether or not they included the treatment effect and its interaction with species and/or time. "Y" and "N" indicate that the effect in the column name was included and not included in the best model of the response variable, respectively. "Y" in the bold font and grey shade indicated that the effect had a 95% confidence interval that did not overlap with zero. The best models of photochemical efficiency, photosynthesis yield, and lenticel density on main stems did not include any of the treatment effect. None of the best models included a three-way interaction effect between treatment, species, and time. "RGR" is the abbreviation of relative growth rates.

Responses	Treatment	Treatment: Species	Treatment: Time
$RGR_{basal.area}$	**Y**	N	Y
RGR_{height}	Y	N	**Y**
$RGR_{stem.volume}$	**Y**	N	**Y**
Chlorophyll content	Y	**Y**	Y
Stomatal conductance	Y	N	Y
Photosynthesis rate	Y	N	N
Predawn leaf water potential	Y	N	N
Non-photochemical quenching	Y	N	N
Lenticel density on young branches	Y	N	**Y**

Table 3.3 Results of ANOVA of total biomass and shoots to roots ratio. D.F. is the abbreviation of degree of freedom. Bold font indicates that the effect was significant (P<0.05).

Source	Total biomass			Shoots to roots ratio		
	D.F.	F ratio	P	D.F.	F ratio	P
Species	3	11.1	**<0.001**	3	13.5	**<0.001**
Treatment	1	4.2	**0.05**	1	26.4	**<0.001**
Species by Treatment	3	1.2	0.33	3	3.7	**0.02**

Figure 3.1 Effects of flooding treatment on relative basal area (left), height (middle), and stem volume (right) growth rates between the flooding and the control treatment. Yellow and blue points indicated average relative growth rates across all species in the control and the flooding treatment, respectively. Error bars were constructed with one standard error of the means.

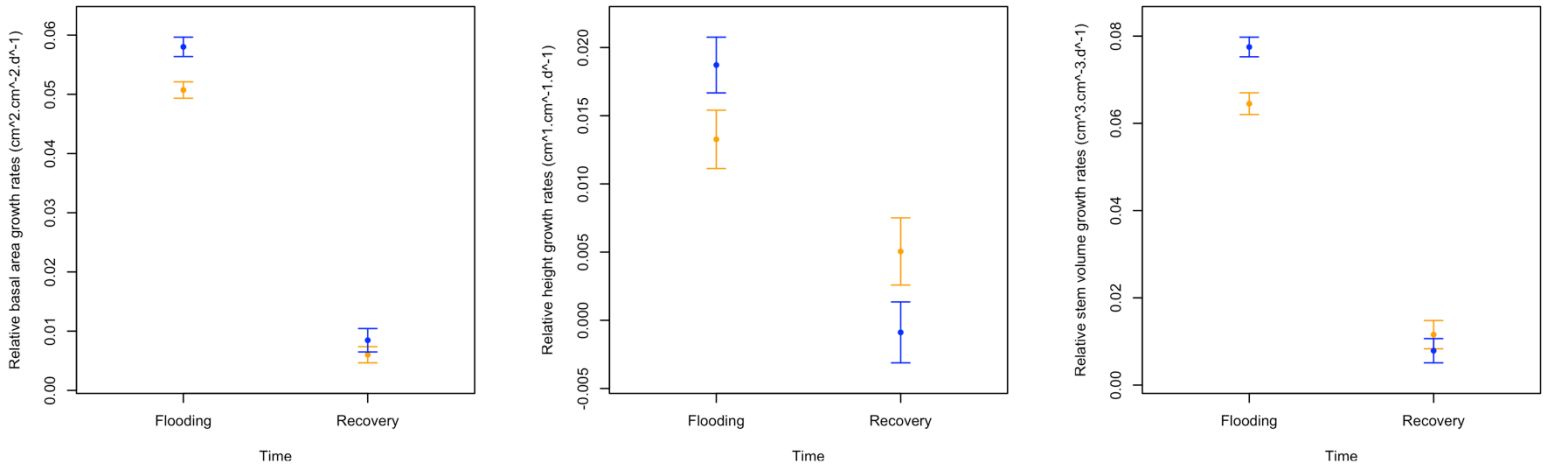


Figure 3.2 Responses of physiological and morphological traits to the flooding treatment. The flooding treatment increased photosynthetic rates (a) and predawn leaf water potential (b), and decreased non-photochemical quenching (NPQ, panel c). There was a significant time by treatment interaction on stomatal conductance (d) and lenticel density on young branches (LDy, panel e). There was a significant species by treatment interaction effect on chlorophyll content (f). Error bars are constructed using one standard error of the means. Yellow and blue indicated the control and the flooding treatment, respectively. Species names were indicated by first three letters.

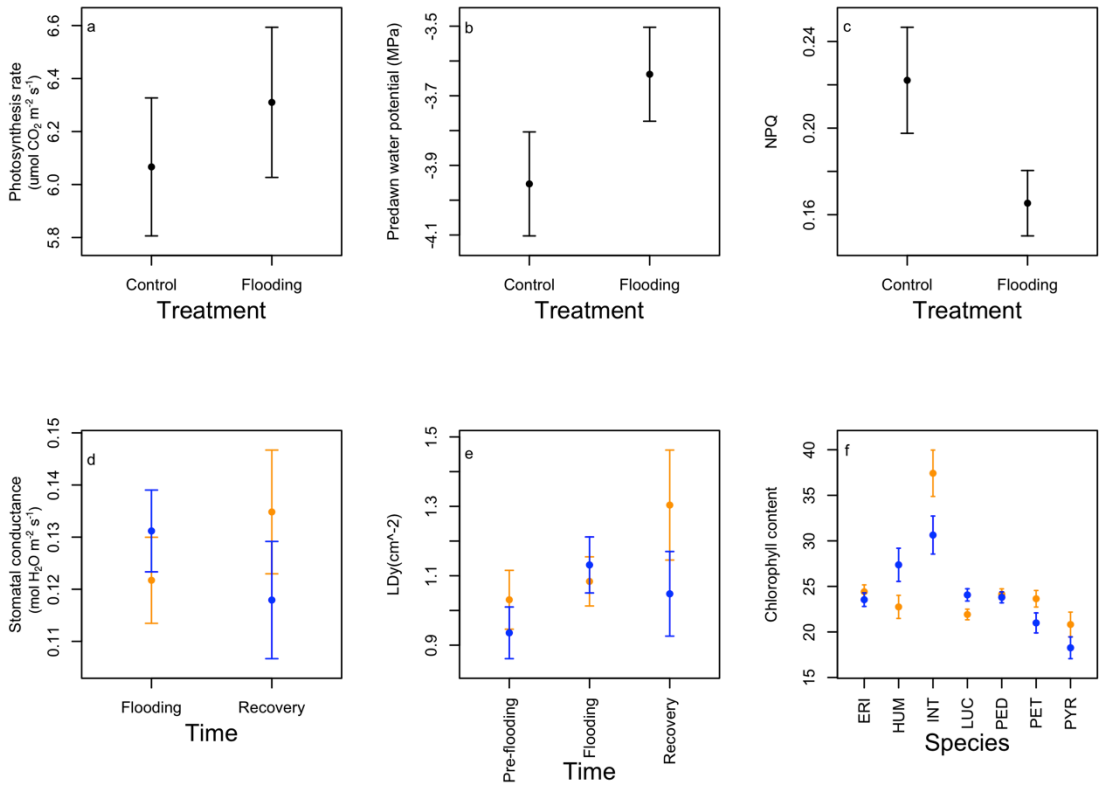


Figure 3.3 Responses of total biomass and allocation to the flooding treatment. (a) Flooding treatment increased the total biomass in the four harvested species. (b) There was a species by treatment interaction effect on shoots to roots ratio. (c) The proportion of biomass allocated to adventitious roots was different between species. Error bars were constructed using one standard error of the means. Yellow and blue indicate the control and the flooding treatment, respectively. Species names were indicated with first three letters.

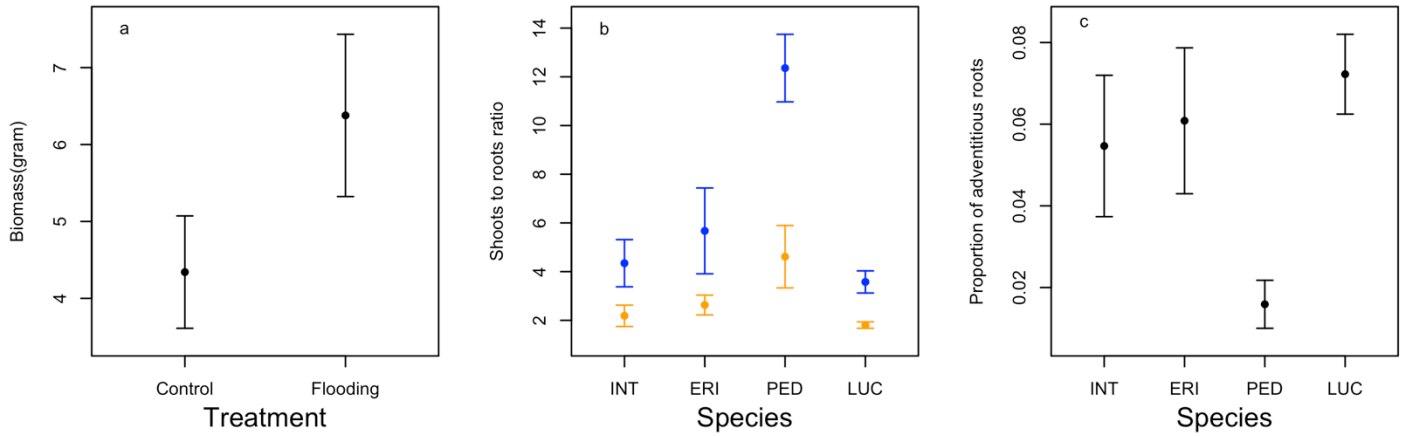
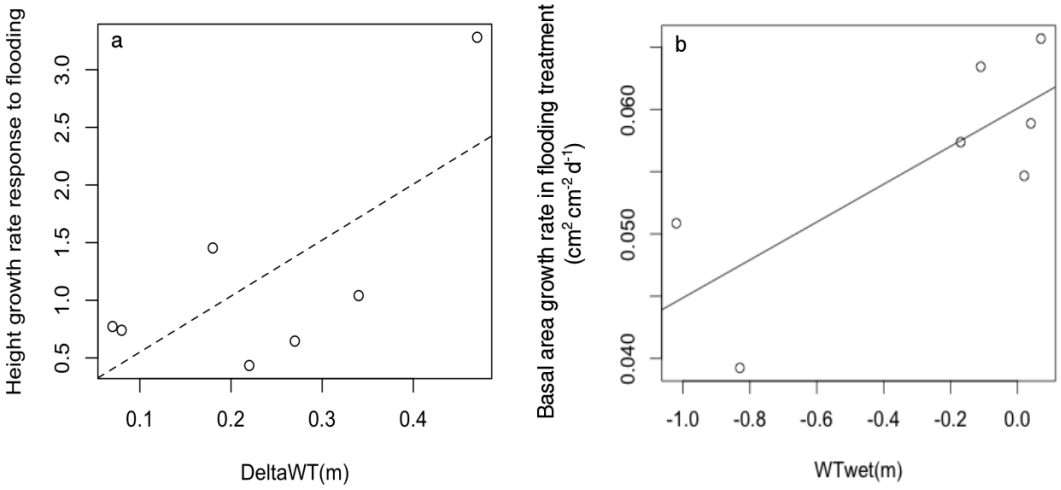


Figure 3.4 Species growth responses to the flooding treatment on their distributions along hydrologic gradients. (a) Variation of stem height growth rate in the flooding treatment between the flooding and the control period was marginally significantly correlated with growing season fluctuation of water table (DeltaWT) in species' natural habitats (r=0.71, P=0.07). (b) Basal area growth rate in the flooding treatment was positively correlated with average growing season maximum water table depth in species' natural habitats (r=0.77, P=0.04). Solid and dash lines are the significant and insignificant least square regression lines.



Bibliography

Ackerly, D. D., S. A. Dudley, S. E. Sultan, J. Schmitt, J. S. Coleman, C. R. Linder, D. R. Sandquist, M. A. Geber, A. S. Evans, and T. E. Dawson. 2000. The evolution of plant ecophysiological traits: recent advances and future directions. *Bioscience* **50**(11): 979-995.

Ackerly DD. 2003. Community assembly, niche conservatism, and adaptive evolution in changing environments. *International Journal of Plant Sciences* **164**(3): S165-S184.

Agrawal, Anurag A., and Mark Fishbein. 2006. Plant defense syndromes. *Ecology* **87**(7): S132-S149.

Agrawal AA, Weber MG. 2015. On the study of plant defence and herbivory using comparative approaches: how important are secondary plant compounds. *Ecology Letters* **18**(10): 985-991.

Akaike, H., 1974. A new look at the statistical model identification. *IEEE transactions on automatic control* **19**(6): 716-723.

Amlin NM, Rood SB. 2002. Comparative tolerances of riparian willows and cottonwoods to water-table decline. *Wetlands* **22**(2): 338-346.

Araya YN, Silvertown J, Gowing DJ, McConway KJ, Linder HP, Midgley G. 2011. A fundamental, eco-hydrological basis for niche segregation in plant communities. *New Phytologist* **189**(1): 253-258.

Armstrong W. 1968. Oxygen diffusion from the roots of woody species. *Physiologia Plantarum* **21**(3): 539-543.

Armstrong W. 1979. Aeration in higher plants. *Advances in botanical research* **7**: 225-332.

Armstrong W, Brändle R, Jackson M. 1994. Mechanisms of flood tolerance in plants. *Acta Botanica Neerlandica* **43**(4): 307-358.

Ayres MP, Clausen TP, MacLean Jr SF, Redman AM, Reichardt PB. 1997. Diversity of structure and antiherbivore activity in condensed tannins. *Ecology* **78**(6): 1696-1712.

Bailey-Serres, J., and L. Voesenek. 2008. Flooding stress: acclimations and genetic diversity. *Annu.Rev.Plant Biol.* **59**:313-339.

Barbehenn RV, Constabel CP. 2011. Tannins in plant–herbivore interactions. *Phytochemistry* **72**(13): 1551-1565.

Barnett TP, Pierce DW, Hidalgo HG, Bonfils C, Santer BD, Das T, Bala G, Wood AW, Nozawa T, Mirin AA, et al. 2008. Human-induced changes in the hydrology of the western United States. *Science* **319**(5866): 1080-1083.

Bartlett MK, Scoffoni C, Sack L. 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecology Letters* **15**(5): 393-405.

Bazzaz, F. 1991. Habitat selection in plants. **1991.** *American Naturalist* **137**:S116-S130.

Bazzaz FA. 1996. *Plants in changing environments: linking physiological, population, and community ecology*: Cambridge University Press.

Becerra JX. 1997. Insects on plants: macroevolutionary chemical trends in host use. *Science* **276**(5310): 253-256.

Berenbaum MR. 1995. Turnabout is fair play-secondary roles for primary compounds. *Journal of Chemical Ecology* **21**(7): 925-940.

Berenbaum MR, Zangerl AR 1997. Adaptation or random variation? In: Romero J, Saunders JA, Barbosa P eds. *Recent advances in phytochemistry:Phytochemical diversity and redundancy in ecological interactions*. New York: Plenum Publishing Corporation, 1-24.

Blomberg SP, Garland T, Ives AR. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* **57**(4): 717-745.

Boeckler GA, Gershenzon J, Unsicker SB. 2011. Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. *Phytochemistry* **72**(13): 1497-1509.

Bréda, N., R. Huc, A. Granier, and E. Dreyer. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* **63**:625-644.

Brenes-Arguedas T, Coley PD, Kursar TA. 2009. Pests vs. drought as determinants of plant distribution along a tropical rainfall gradient. *Ecology* **90**(7): 1751-1761.

Bryant JP, Reichardt P, Clausen T, Werner R. 1993. Effects of mineral nutrition on delayed inducible resistance in Alaska paper birch. *Ecology* **74**(7): 2072-2084.

Cacho, N.I., Kliebenstein, D.J. and Strauss, S.Y., 2015. Macroevolutionary patterns of glucosinolate defense and tests of defense escalation and resource availability hypotheses. *New Phytologist*, **208**(3):915-927.

Callaway, R. M., R. Brooker, P. Choler, Z. Kikvidze, C. J. Lortie, R. Michalet, L. Paolini, F. I. Pugnaire, B. Newingham, and E. T. Aschehoug. 2002. Positive interactions among alpine plants increase with stress. *Nature* **417** (6891):844-848.

Castagneyrol B, Jactel H, Vacher C, Brockerhoff EG, Koricheva J. 2014. Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. *Journal of applied ecology* **51**(1): 134-141.

Catford JA, Jansson R. 2014. Drowned, buried and carried away: effects of plant traits on the distribution of native and alien species in riparian ecosystems. *New Phytologist* **204**(1): 19-36.

Cavender-Bares J, Ackerly DD, Baum DA, Bazzaz FA. 2004. Phylogenetic overdispersion in Floridian oak communities. *The American Naturalist* **163**(6): 823-843.

Cavender-Bares J, Kitajima K, Bazzaz F. 2004. Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. *Ecological Monographs* **74**(4): 635-662.

Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW. 2009. The merging of community ecology and phylogenetic biology. *Ecology Letters* **12**(7): 693-715.

Chapin FS, Autumn K, Pugnaire F. 1993. Evolution of suites of traits in response to environmental stress. *American Naturalist* **142**: S78-S92.

Chase JM, Leibold MA. 2003. *Ecological niches: linking classical and contemporary approaches*: University of Chicago Press, Chicago, Illinois, USA.

Chaves, M. M., J. S. Pereira, J. Maroco, M. L. Rodrigues, C. P. Ricardo, M. L. Osorio, I. Carvalho, T. Faria, and C. Pinheiro. 2002. How plants cope with water stress in the field. Photosynthesis and growth. *Annals of botany* **89**:907-916.

Chesson P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* **31**: 343-366.

Coley PD, Barone J. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* **27**: 305-335.

Colmer T. 2003. Long distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell & Environment* **26**(1): 17-36.

Colmer T, Voesenek L. 2009. Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology* **36**(8): 665-681.

Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M., Beckman, N. and Zhu, Y., 2014. Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance and density dependent seed and seedling survival. *Journal of Ecology*, **102**(4): 845-856.

Condit R, Pitman N, Leigh EG, Chave J, Terborgh J, Foster RB, Nunez P, Aguilar S, Valencia R, Villa G, et al. 2002. Beta-diversity in tropical forest trees. *Science* **295**(5555): 666-669.

Cornwell, W. K., and D. D. Ackerly. 2009. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs* **79**(1):109-126.

Craine, J. M., B. M. Engelbrecht, C. H. Lusk, N. G. McDowell, and H. Poorter. 2012. Resource limitation, tolerance, and the future of ecological plant classification. *Frontiers in plant science* **3**:1-10.

Crane J, Davies F. 1989. Flooding responses of Vaccinium species. *HortScience* **24**(2): 203-210.

Crawford, R.M.M. and Braendle, R., 1996. Oxygen deprivation stress in a changing environment. *Journal of Experimental Botany*, **47**(2):145-159.

Crawley MJ. 1989. Insect herbivores and plant population dynamics. *Annual review of entomology* **34**(1): 531-562.

Darwin C 1859. On the ongin of species: London: John Murray.

Davies FS, Flore JA. 1986. Short-term flooding effects on gas exchange and quantum yield of rabbiteye blueberry (*Vaccinium ashei Reade*). *Plant Physiology* **81**(1): 289-292.

De Steven, D. 1991. Experiments on mechanisms of tree establishment in old-field succession: seedling survival and growth. *Ecology* **72**(3):1076-1088.

Diaz, S., M. Cabido, and F. Casanoves. 1998. Plant functional traits and environmental filters at a regional scale. *Journal of vegetation science* **9** (1):113-122.

Dickson RE, Broyer T, Johnson C. 1972. Nutrient uptake by tupelo gum and bald cypress from saturated or unsaturated soil. *Plant and Soil* **37**(2): 297-308.

Dyer LA, Singer M, Lill J, Stireman J, Gentry G, Marquis RJ, Ricklefs R, Greeney HF, Wagner DL, Morais H. 2007. Host specificity of Lepidoptera in tropical and temperate forests. *Nature* **448**(7154): 696-699.

Ehrlén, J. and Eriksson, O., 2000. Dispersal limitation and patch occupancy in forest herbs. *Ecology,* **81**(6):1667-1674.

Emery NC, Stanton ML, Rice KJ. 2009. Factors driving distribution limits in an annual plant community. *New Phytologist* **181**(3): 734-747.

Feeny, P., 1976. Plant apparency and chemical defense. In *Biochemical interaction between plants and insects*. New York, Springer, 1-40.

Fine PVA, Mesones I, Coley PD. 2004. Herbivores promote habitat specialization by trees in amazonian forests. *Science* **305**(5684): 663-665.

Fine PVA, Metz MR, Lokvam J, Mesones I, Zuniga JMA, Lamarre GPA, Pilco MV, Baraloto C. 2013. Insect herbivores, chemical innovation, and the evolution of habitat specialization in Amazonian trees. *Ecology* **94**(8): 1764-1775.

Futuyma DJ, Agrawal AA. 2009. Macroevolution and the biological diversity of plants and herbivores. *Proceedings of the National Academy of Sciences* **106**(43): 18054-18061.

Gerhold, P., Cahill, J.F., Winter, M., Bartish, I.V. and Prinzing, A., 2015. Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). *Functional Ecology*, **29**(5): 600-614.

Geyer CJ, Wagenius S, Shaw RG. 2007. Aster models for life history analysis. *Biometrika* **94**(2): 415-426.

Goulden, M. L. 1996. Carbon assimilation and water-use efficiency by neighboring Mediterranean-climate oaks that differ in water access. *Tree physiology* **16**(4):417-424.

Grigal DF, Homann PS. 1994. Nitrogen mineralization, groundwater dynamics, and forest growth on a Minnesota outwash landscape. *Biogeochemistry* **27**(3): 171-185.

Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**(4): 457-461.

Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**(1): 129-131.

Hirose, T., and M. J. Werger. 1995. Canopy structure and photon flux partitioning among species in a herbaceous plant community. *Ecology* **76** (2):466-474.

Hosner JF, Leaf AL. 1962. The effect of soil saturation upon the dry weight, ash content, and nutrient absorption of various bottomland tree seedlings. *Soil Science Society of America Journal* **26**(4): 401-404.

Huner, N. P., G. Öquist, V. M. Hurry, M. Krol, S. Falk, and M. Griffith. 1993. Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. *Photosynthesis Research* **37**(1):19-39.

Huntington TG. 2006. Evidence for intensification of the global water cycle: Review and synthesis. *Journal of Hydrology* **319**(1-4): 83-95.

Huntly N. 1991. Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics* **22**: 477-503.

Hutchinson GE. 1957. Cold spring harbor symposium on quantitative biology. *Concluding remarks* **22**: 415-427.

Hurvich, C.M. and Tsai, C.L., 1991. Bias of the corrected AIC criterion for underfitted regression and time series models. *Biometrika*, **78**(3): 499-509.

Jabot, F. and Bascompte, J., 2012. Bitrophic interactions shape biodiversity in space. *Proceedings of the National Academy of Sciences*, **109**(12): 4521-4526.

Jackson M, Drew M. 1984. Effects of flooding on growth and metabolism of herbaceous plants. *Flooding and plant growth* **1**: 47-128.

Jackson MB, Attwood PA 1997. Roots of willow (*Salix viminalis L.*) show marked tolerance to oxygen shortage in flooded soils and in solution culture. *Plant Roots-From Cells to Systems*: New York, Springer, 37-45.

Johnson, M.T., Ives, A.R., Ahern, J. and Salminen, J.P., 2014. Macroevolution of plant defenses against herbivores in the evening primroses. *New Phytologist*, **203**(1): 267-279.

Julkunen-Tiitto R, Rousi M, Bryant J, Sorsa S, Keinänen M, Sikanen H. 1996. Chemical diversity of several Betulaceae species: comparison of phenolics and terpenoids in northern birch stems. *Trees* **11**(1): 16-22.

Kelly CA, Dyer RJ. 2002. Demographic consequences of inflorescence-feeding insects for Liatris cylindracea, an iteroparous perennial. *Oecologia* **132**(3): 350-360.

Kobe RK. 1999. Light gradient partitioning among tropical tree species through differential seedling mortality and growth. *Ecology* **80**(1): 187-201.

Koricheva, J., Larsson, S., Haukioja, E. and Keinänen, M., 1998. Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos* **83**(2): 212-226.

Kozlowski T. 1997. Responses of woody plants to flooding and salinity. *Tree physiology monograph* **1**(1): 1-29.

Kraft, N.J., Comita, L.S., Chase, J.M., Sanders, N.J., Swenson, N.G., Crist, T.O., Stegen, J.C., Vellend, M., Boyle, B., Anderson, M.J. and Cornell, H.V., 2011. Disentangling the drivers of β diversity along latitudinal and elevational gradients. *Science*, **333**(6050):1755-1758.

Kraft NJ, Godoy O, Levine JM. 2015. Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences* **112**(3): 797-802.

Kursar, T.A., Dexter, K.G., Lokvam, J., Pennington, R.T., Richardson, J.E., Weber, M.G., Murakami, E.T., Drake, C., McGregor, R. and Coley, P.D., 2009. The

evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus Inga. *Proceedings of the National Academy of Sciences*, **106**(43): 18073-18078.

Laughlin, D.C. and Messier, J., 2015. Fitness of multidimensional phenotypes in dynamic adaptive landscapes. *Trends in Ecology & Evolution*, **30**(8): 487-496.

Legendre P, Borcard D, Peres-Neto PR. 2005. Analyzing beta diversity: Partitioning the spatial variation of community composition data. *Ecological Monographs* **75**(4): 435-450.

Lind EM, Vincent JB, Weiblen GD, Cavender-Bares J, Borer ET. 2015. Trophic phylogenetics: evolutionary influences on body size, feeding, and species associations in grassland arthropods. *Ecology* **96**(4): 998-1009.

Lindroth R, Peterson S. 1988. Effects of plant phenols of performance of southern armyworm larvae. *Oecologia* **75**(2): 185-189.

Lindroth RL. 1988. Hydrolysis of phenolic glycosides by midgut β-glucosidases in Papilio glaucus subspecies. *Insect biochemistry* **18**(8): 789-792.

Lindroth RL, Hwang SY 1997. Diversity, redundancy, and multiplicity in chemical defense system of aspen. In: Romero J, Saunders JA, Barbosa P eds. *Recent advances in phytochemistry: Phytochemical diversity and redundancy in ecological interactions*. New York: Plenum Publishing Corporation, 25-58.

Lindroth RL, Kinney KK, Platz CL. 1993. Responses of diciduous trees to elevated atmospheric CO2: productivity, phytochemistry, and insect performance. *Ecology* **74**(3): 763-777.

Losos, J.B., 2011. Convergence, adaptation, and constraint. *Evolution* **65**(7):1827-1840.

Louda SM. 1982. Distribution ecology: variation in plant recruitment over a gradient in relation to insect seed predation. *Ecological Monographs* **52**(1): 25-41.

Louda SM. 1983. Seed predation and seedling mortality in the recruitment of a shrub, Haplopappus venetus (Asteraceae), along a climatic gradient. *Ecology* **64**(3): 511-521.

Madritch MD, Lindroth RL. 2015. Condensed tannins increase nitrogen recovery by trees following insect defoliation. *New Phytologist* **208**(2): 410-420.

Maherali, H., Pockman, W.T. and Jackson, R.B., 2004. Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology*, **85**(8): 2184-2199.

Maherali, H. and DeLucia, E.H., 2000. Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. *Tree Physiology*, **20**(13): 859-867.

Marks, C.O. and Lechowicz, M.J., 2006. Alternative designs and the evolution of functional diversity. *The American Naturalist*, **167**(1) :55-66.

Maron JL, Crone E. 2006. Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society of London B: Biological Sciences* **273**(1601): 2575-2584.

Matsuki M, MacLean Jr SF. 1994. Effects of different leaf traits on growth rates of insect herbivores on willows. *Oecologia* **100**(1-2): 141-152.

McKevlin M, Hook D, McKee JW. 1995. Growth and nutrient use efficiency of water tupelo seedlings in flooded and well-drained soil. *Tree Physiology* **15**(11): 753-758.

Matsuki, M. and MacLean Jr, S.F., 1994. Effects of different leaf traits on growth rates of insect herbivores on willows. *Oecologia,* **100**(1-2):141-152.

Mittelbach GG, Schemske DW, Cornell HV, Allen AP, Brown JM, Bush MB, Harrison SP, Hurlbert AH, Knowlton N, Lessios HA. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters* **10**(4): 315-331.

Monk, L.S., Fagerstedt, K.V. and Crawford, R.M., 1987. Superoxide Dismutase as an Anaerobic Polypeptide A Key Factor in Recovery from Oxygen Deprivation in Iris pseudacorus? *Plant Physiology*, **85**(4):1016-1020.

Narwani A, Matthews B, Fox J, Venail P. 2015. Using phylogenetics in community assembly and ecosystem functioning research. *Functional Ecology* **29**(5): 589-591.

Ness J, Rollinson E, Whitney K. 2011. Phylogenetic distance can predict susceptibility to attack by natural enemies. *Oikos* **120**(9): 1327-1334.

Novotny V, Basset Y. 2005. Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society of London B: Biological Sciences* **272**(1568): 1083-1090.

Orians CM, Huang CH, Wild A, Dorfman KA, Zee P, Dao MTT, Fritz RS. 1997. Willow hybridization differentially affects preference and performance of herbivorous beetles. *Entomologia Experimentalis Et Applicata* **83**(3): 285-294.

Paradis, E., Claude, J. and Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics,* **20**(2): 289-290.

Pearse IS, Hipp AL. 2009. Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences* **106**(43): 18097-18102.

Pezeshki S. 1994. Plant response to flooding. *Plant-environment interactions. New York, M. Dekker*, 289-321.

Porter LJ, Hrstich LN, Chan BG. 1985. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **25**(1): 223-230.

Qian, H., 2009. Global comparisons of beta diversity among mammals, birds, reptiles, and amphibians across spatial scales and taxonomic ranks. Journal of Systematics and *Evolution*, **47**(5): 509-514.

Qian, H. and Ricklefs, R.E., 2007. A latitudinal gradient in large scale beta diversity for vascular plants in North America. *Ecology letters,* **10**(8):737-744.

Rasmann, S. and Agrawal, A.A., 2011. Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. *Ecology Letters*, **14**(5): 476-483.

R Core Team 2016. R: A language and environment for statistical computing: R Foundation for Statistical Computing.

Reich PB. 2014. The worldwide 'fast–slow'plant economics spectrum: a traits manifesto. *Journal of Ecology* **102**(2): 275-301.

Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB. 2003. The evolution of plant functional variation: Traits, spectra, and strategies. *International Journal of Plant Sciences* **164**(3): S143-S164.

Revell, L.J., 2009. Size correction and principal components for interspecific comparative studies. *Evolution* **63**(12): 3258-3268.

Riggs CE, Hobbie SE, Savage JA, Cavender-Bares J, Wei X. 2015. Contrasting effects of plant species traits and moisture on the decomposition of multiple litter fractions. *Oecologia.* **179**(2): 573-584.

Sargent, R.D. and Ackerly, D.D., 2008. Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology & Evolution*, **23**(3): 123-130.

Savage J. 2010. *An ecological and evolutionary perspective on functional diversity in the genus Salix.* Ph.D., University of Minnesota, USA.

Savage J, Cavender-Bares JM. 2012. Habitat specialization and the role of trait lability in structuring diverse willow (genus: *Salix*) communities. *Ecology* **93**(8): S138-S150.

Savage JA, Cavender-Bares J. 2013. Phenological cues drive an apparent trade-off between freezing tolerance and growth in the family Salicaceae. *Ecology* **94**(8): 1708-1717.

Savage JA, Cavender-Bares J, Verhoeven A. 2009. Willow species (genus: *Salix*) with contrasting habitat affinities differ in their photoprotective responses to water stress. *Functional Plant Biology* **36**(4): 300-309.

Savage JA, Cavender-Bares JM. 2011. Contrasting drought survival strategies of sympatric willows (genus: *Salix*): consequences for coexistence and habitat specialization. *Tree Physiology* **31**(6): 604-614.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Meth* **9**(7): 671-675.

Schroeder-Georgi, T., Wirth, C., Nadrowski, K., Meyer, S.T., Mommer, L. and Weigelt, A., 2016. From pots to plots: hierarchical trait based prediction of plant performance in a mesic grassland. *Journal of Ecology*, **104**(1): 206-218.

Schwilk, D.W. and Ackerly, D.D., 2001. Flammability and serotiny as strategies: correlated evolution in pines. *Oikos*, **94**(2): 326-336.

Seeley M 2012. Meterologic Measurements at Cedar Creek Natural History Area - Experiment 080.

Shaw RG, Geyer CJ, Wagenius S, Hangelbroek HH, Etterson JR. 2008. Unifying life-history analyses for inference of fitness and population growth. *American Naturalist* **172**(1): E35-E47.

Sherman RE, Fahey TJ, Howarth RW. 1998. Soil-plant interactions in a neotropical mangrove forest: iron, phosphorus and sulfur dynamics. *Oecologia* **115**(4): 553-563.

Silvertown J, Araya Y, Gowing D. 2015. Hydrological niches in terrestrial plant communities: a review. *Journal of Ecology* **103**(1): 93-108.

Silvertown J, Dodd ME, Gowing DJG, Mountford JO. 1999. Hydrologically defined niches reveal a basis for species richness in plant communities. *Nature* **400**(6739): 61-63.

Sterck, F., Markesteijn, L., Schieving, F. and Poorter, L., 2011. Functional traits determine trade-offs and niches in a tropical forest community. *Proceedings of the National Academy of Sciences*, **108**(51): 20627-20632.

Sterck, F., Markesteijn, L., Toledo, M., Schieving, F. and Poorter, L., 2014. Sapling performance along resource gradients drives tree species distributions within and across tropical forests. *Ecology*, **95(**9): 2514-2525.

Striker G, Insausti P, Grimoldi A, Leon R. 2006. Root strength and trampling tolerance in the grass Paspalum dilatatum and the dicot Lotus glaber in flooded soil. *Functional Ecology* **20**(1): 4-10.

Striker G, Insousti P, Grimoldi A. 2007. Effects of flooding at early summer on plant water relations of *Lotus tenuis*. *Lotus Newsl* **37**(1): 1-7.

Sugiura, N., 1978. Further analysts of the data by akaike's information criterion and the finite corrections. *Communications in Statistics-Theory and Methods*, **7**(1):13-26.

Swenson, N.G. and Enquist, B.J., 2009. Opposing assembly mechanisms in a Neotropical dry forest: implications for phylogenetic and functional community ecology. *Ecology*, **90**(8):2161-2170.

T**ilman D. 1982.** *Resource competition and community structure*: Princeton University Press, Princeton, NJ.

Tuomisto H, Ruokolainen K, Kalliola R, Linna A, Danjoy W, Rodriguez Z. 1995. Dissecting Amazonian diversity. *Science* **269**(5220): 63-66.

Tuomisto, H., Ruokolainen, K., Aguilar, M. and Sarmiento, A., 2003. Floristic patterns along a 43 km long transect in an Amazonian rain forest. *Journal of Ecology*, **91**(5): 743-756.

Tyree MT, Ewers FW. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**(3): 345-360.

Ushimaru, T., Shibasaka, M. and Tsuji, H., 1992. Development of the-detoxification system during adaptation to air of submerged rice seedlings. *Plant and cell physiology*, **33**(8):1065-1071.

Van der Valk, A.G., 1981. Succession in wetlands: a gleasonian appraoch. *Ecology*, **62**(3): 688-696.

Vartapetian BB, Jackson MB. 1997. Plant adaptations to anaerobic stress. *Annals of Botany* **79**: 3-20.

Viola DV, Mordecai EA, Jaramillo AG, Sistla SA, Albertson LK, Gosnell JS, Cardinale BJ, Levine JM. 2010. Competition–defense tradeoffs and the maintenance of plant diversity. *Proceedings of the National Academy of Sciences* **107**(40): 17217-17222.

Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. and Garnier, E., 2007. Let the concept of trait be functional! *Oikos*, **116**(5): 882-892.

Voesenek L, Rijnders J, Peeters A, Van de Steeg H, De Kroon H. 2004. Plant hormones regulate fast shoot elongation under water: from genes to communities. *Ecology* **85**(1): 16-27.

Volf M, Hrcek J, Julkunen-Tiitto R, Novotny V. 2015a. To each its own: differential response of specialist and generalist herbivores to plant defence in willows. *Journal of Animal Ecology* **84**(23):1123-1132.

Volf M, Julkunen-Tiitto R, Hrcek J, Novotny V. 2015b. Insect herbivores drive the loss of unique chemical defense in willows. *Entomologia Experimentalis Et Applicata* **156**(1): 88-98.

Warmings E. 1895. *Plantesamfund-Grundtrack af den okologiske Plantegeografi.* P.G. Philipsens Forlog, Copenhagen.

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* **33**: 475-505.

Weber, M. and Brändle, R., 1996. Some aspects of the extreme anoxia tolerance of the sweet flag, Acorus calamus L. *Folia Geobotanica*, **31**(1): 37-46.

Weiblen GD, Webb CO, Novotny V, Basset Y, Miller SE. 2006. Phylogenetic dispersion of host use in a tropical insect herbivore community. *Ecology* **87**(sp7): S62-S75.

Weiher, E. and Keddy, P.A., 1995. The assembly of experimental wetland plant communities. *Oikos* **73**(3): 323-335.

Whittaker, R.H., 1960. Vegetation of the Siskiyou mountains, Oregon and California. *Ecological monographs*, **30**(3): 279-338.

Whittaker, R.H., 1972. Evolution and measurement of species diversity.*Taxon* **21**(2-3): 213-251.

Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V., Damschen, E.I., Jonathan Davies, T., Grytnes, J.A., Harrison, S.P. and Hawkins, B.A., 2010. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology letters* **13**(10):1310-1324.

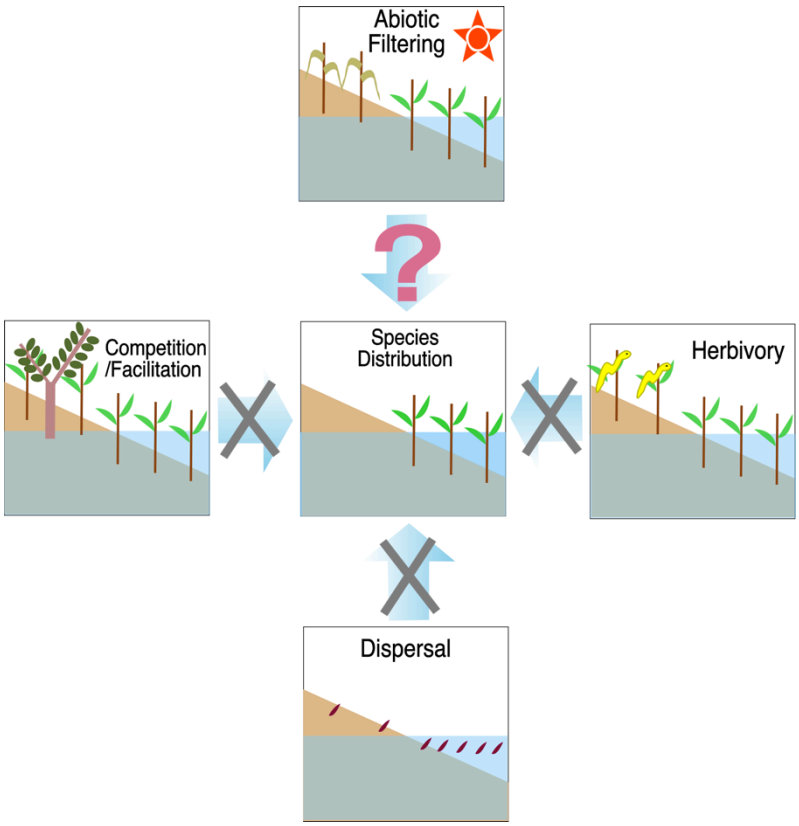
Woodward, F.I. and Diament, A.D., 1991. Functional approaches to predicting the ecological effects of global change. *Functional Ecology* **5**(2): 202-212.

Yordanova RY, Christov KN, Popova LP. 2004. Antioxidative enzymes in barley plants subjected to soil flooding. *Environmental and Experimental Botany* **51**(2): 93-101.

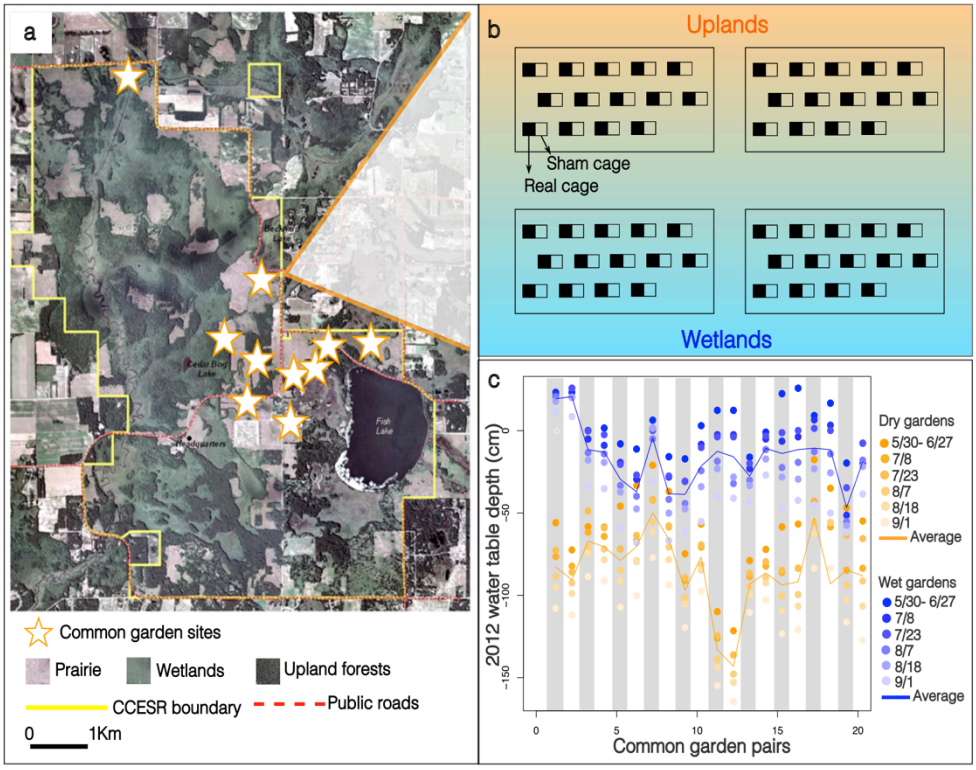
Young B, Wagner D, Doak P, Clausen T. 2010. Induction of phenolic glycosides by quaking aspen (Populus tremuloides) leaves in relation to extrafloral nectaries and epidermal leaf mining. *Journal of Chemical Ecology* **36**(4): 369-377.

Appendices

Appendix 1 Conceptual figure of chapter 1. Testing environmental filtering using an experimental approach. Multiple community assembly processes could cause variation of species distributions along environmental gradients. The field experimental approach allowed us to test the effect of environmental filtering on species distributions along hydrologic gradients while controlling for three alternative community assembly processes, which were dispersal, neighborhood interactions, and herbivory.



Appendix 2 (a) Aerial photo of Cedar Creek Ecosystem Science Reserve. The reserve has a mosaic landscape consisting of patches of wetlands, prairie, and upland forests, forming natural hydrologic gradients. We established 40 common gardens at 10 sites within the reserve. **(b) The design of common gardens at one site.** Two gardens were located in a wetland; the other two were located in the adjacent upland. Each gardens had 28 cuttings, two from each of the 14 species. One of the two conspecific plants was enclosed in a real insect exclusion cage; the other plant was enclosed in a sham cage. The gardens were surrounded with metal fences to exclude large mammalian herbivores. To reduce competition and other neighborhood interactions, plants were 1.4 m away from each other, and a piece of 1m by 1m landscape fabric was installed beneath each plant to suppress native vegetation (not shown in the figure). **(c) Water table depth in common gardens during 2012 growing season.**



Appendix 3. Species hydrologic niches determined by their natural abundances along water table depth gradients based on Savage & Cavender-Bares (2012). WT_{dry} and WT_{wet} are the water table depth weighted by species abundance during the driest (August) and wettest (May) month of the growing season.

Species	**Abbreviation**	**WT_{dry} (cm)**	**WT_{wet} (cm)**
S. interior	INT	-110	-102
S. humilis	HUM	-117	-83
P. deltoides	DEL	-86	-77
S. amygdaloides	AMY	-40	-19
S. eriocephala	ERI	-62	-18
S. petiolaris	PET	-43	-15
S. bebbiana	BEB	-46	-18
S. discolor	DIS	-50	-3
S. nigra	NIG	-24	-1
S. candida	CAN	-30	1
S. pyrifolia	PYR	-4	1
S. serissima	SER	-20	2
S. pedicellaris	PED	-14	4
S. lucida	LUC	0	7

Appendix 4. Functional traits, their associations with water transport or water stress tolerance, and their Pearson correlations with species hydrologic niche and water table depth optima. Correlations coefficients in bold and italic fonts are significant ($p<0.05$) and marginally significant ($p<0.1$), respectively.

Traits	Abbreviations	Associations with water transport or water stress tolerance	Predicted changes from dry to wet habitats	Correlation coefficients with hydrologic niche	Correlation coefficients with water table depth optimum
Turgor loss point	TLP	Plants with a lower TLP are able to maintain leaf turgor at more negative water potentials. Species TLP was positively correlated with water availability in their environments (Bartlett et al. 2012).	Increase	**0.60**	**0.57**
Wood density	WD	Plants with a higher WD can resist xylem cavitation under more negative pressures and therefore are more tolerant to drought (Hacke et al. 2001).	Decrease	-0.33	-0.15
Root elongation rate	RER	A faster RER can help riparian plants to keep up with declining water tables during seasonal drought (Stella & Battles 2010).	Decrease	**-0.65**	**-0.62**
Stomatal pore index	SPI	SPI was positively correlated with leaf conductance; a higher leaf conductance may improve drought tolerance (Sack et al. 2003).	Decrease	**-0.34**	***-0.5***

Traits	Abbreviations	Associations with water transport or water stress tolerance	Predicted changes from dry to wet habitats	Correlation coefficients with hydrologic niche	Correlation coefficients with water table depth optimum
Lenticel density on main stems	LD_b	Lenticels are entry points of oxygen in flooded plants (Armstrong 1968). We hypothesized higher lenticel densities may improve flooding tolerance.	Increase	-0.37	-0.27
Lenticel density on young branches	LD_y			**-0.75**	**-0.60**
Stomatal conductance	g_s	A lower g_s may reduce water loss and therefore improve drought tolerance, but with a cost of decreasing A_{max} due to carbon limitation (Farquhar & Sharkey 1982).	Increase	**-0.67**	**-0.64**
Maximum photosynthetic rate	A_{max}		Increase	**-0.75**	-0.35
Stem specific hydraulic conductivity	K_s	K_s has been found to be higher in species adapted to arid climates (Maherali et al 2004).	Decrease	**-0.52**	**-0.57**

References in Appendix 4

Armstrong, W. 1968. Oxygen diffusion from the roots of woody species. Physiologia Plantarum 21:539-543.

Bartlett, M. K., C. Scoffoni, and L. Sack. 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecology Letters 15:393-405.

Farquhar, G. D., and T. D. Sharkey. 1982. Stomatal conductance and photosynthesis. Annual review of plant physiology 33:317-345.

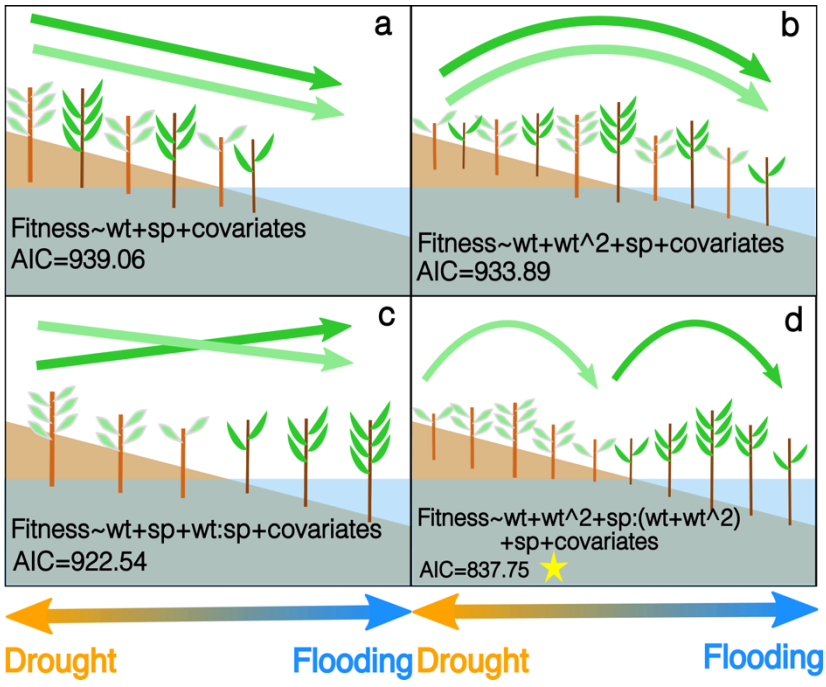
Hacke, U. G., J. S. Sperry, W. T. Pockman, S. D. Davis, and K. A. McCulloh. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia 126:457-461.

Maherali, H., W. T. Pockman, and R. B. Jackson. 2004. Adaptive variation in the vulnerability of woody plants to xylem cavitation. Ecology 85:2184-2199.

Sack, L., P. Cowan, N. Jaikumar, and N. Holbrook. 2003. The 'hydrology'of leaves: co-ordination of structure and function in temperate woody species. Plant, Cell & Environment 26:1343-1356.

Stella, J. C., and J. J. Battles. 2010. How do riparian woody seedlings survive seasonal drought? Oecologia 164:579-590.

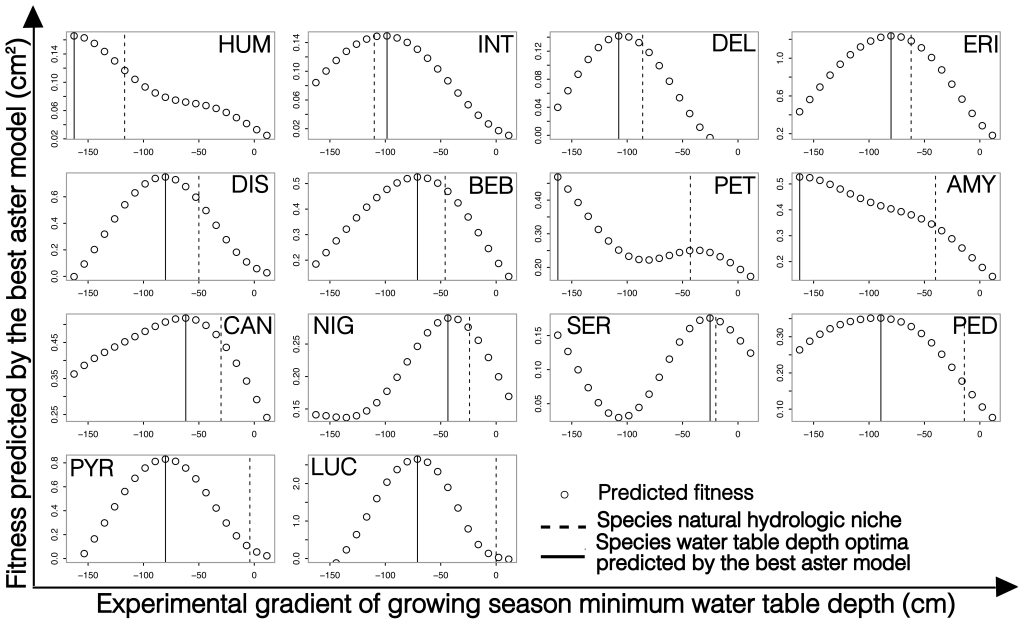
Appendix 5. Four alternative aster fitness models. Along a water table depth gradient, intensities of drought and flooding stress vary in opposite directions. Model (a) assumed that species were sensitive to either drought or flooding but not both, and species fitness varied linearly along the experimental hydrologic gradient. Model (a) also assumed there was no species by water table depth interaction effect, which means slopes of fitness responses to water table depth were same among species. Model (b) also assumed species fitness varied linearly along the experimental hydrologic gradient, but it included a species by water table depth interaction effect. Model (c) assumed species were sensitive to both drought and flooding, and species fitness varied in a hump-shaped manner along the experimental hydrologic gradient, with no species by water table depth interaction effect. Model (d) assumed species fitness varied in a hump-shaped manner along the experimental hydrologic gradient, and with species by water table depth interaction effect. We fitted the four models using growing season maximum water table depth and found model (d) had the lowest AIC value.



Appendix 6. AIC values of four alternative aster models. Similar to models constructed using growing season maximum water table depth, model (d) had the lowest AIC value.

Model	**AIC**
a. Linear response to water table depth, no species by water table depth interaction	940
b. Quadratic response to water table depth, no species by water table depth interaction	935
c. Linear response to water table depth, with species by water table depth interaction	924
d. Quadratic response to water table depth, with species by water table depth interaction	901

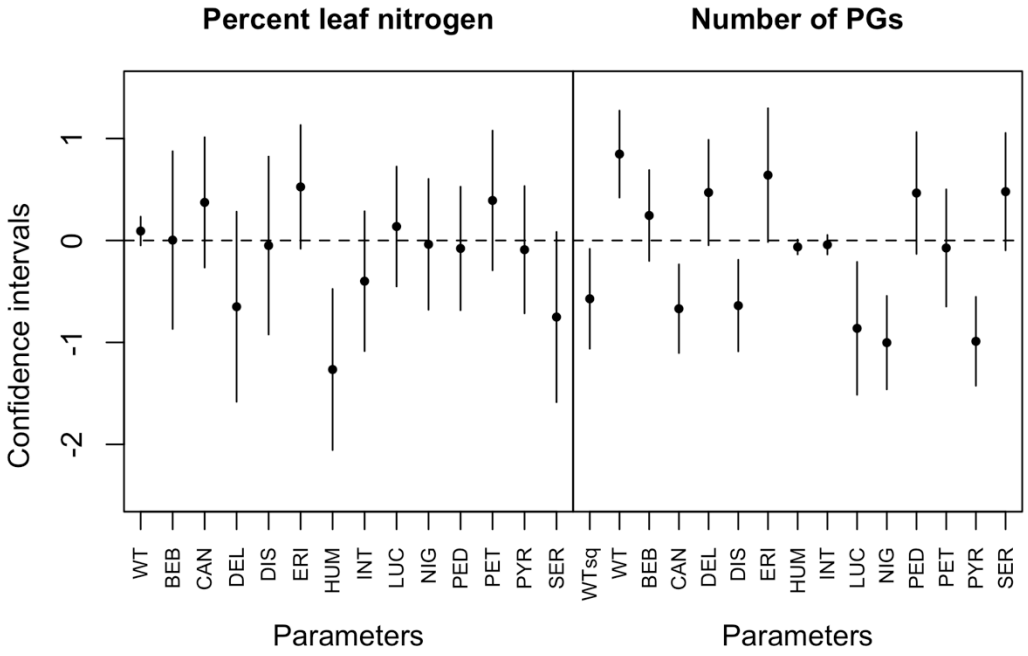
Appendix 7. Species fitness responses along the experimental hydrologic gradient predicted by the best aster fitness model constructed using growing season minimum water table depth. Biomass at the end of the second growing season was used as a surrogate for fitness. A predicted biomass of zero or less indicates the species could not survive at the given water table depth.



Appendix 8. AICc values of the traits models. We compared eight models for each trait. The predictor formulas of these models are indicated in the column names. The AICc values of the best model for each trait are highlighted in grey.

Traits	~1	~sp	~wtd	~wtd+wtd^2	~sp+wtd	~sp+wtd+wtd^2	~sp*wtd	~sp*(wtd+ wtd^2)
Percent leaf nitrogen	553	545	554	555	546	547	566	601
Percent leaf carbon	563	371	560	562	372	374	385	405
CTs concentration	470	291	470	472	294	296	309	342
PGs concentration	710	561	712	713	563	564	572	600
Number of PGs	713	595	715	716	596	596	603	617

Appendix 9 Averaged parameter estimates and 95% confidence intervals for the best models of leaf percent nitrogen, and the number of PGs. Solid dots are the averaged parameter estimates. Solid vertical lines are the 95% confidence intervals of the averaged estimates. Dash horizontal lines indicate zero. "WT" represents water table depth, "WTsq" represent quadratic term of water table depth. Species are indicated with the first three letters of their species names.



Appendix 10. Phylogenetic signals in leaf traits. K_{obs} represents the observed K value, K_{rand} and K_{BM} represent the means of random null K and Brownian null K distributions. Bold and italic fonts indicate significant and marginally significant phylogenetic signals ($P<0.05$ and $P<0.1$), respectively. "sd" is the abbreviation for standard deviation.

Traits	K_{obs}	K_{rand} (sd)	K_{BM}(sd)
Percent leaf nitrogen	0.54	0.35(0.25)	1.02(0.50)
Percent leaf carbon	0.31	0.30(0.14)	1.03(0.55)
CTs concentration	***0.52***	***0.31(0.14)***	0.99(0.51)
PGs concentration	0.39	0.32(0.17)	1.01(0.51)
Number of PGs	**1.79**	**0.30(0.14)**	***0.95(0.50)***
Salicin concentration	**2.09**	**0.34(0.22)**	***1.01(0.52)***
Salicortin concentration	0.44	0.34(0.22)	0.99(0.50)
Tremulacin concentration	0.31	0.36(0.31)	1.01(0.50)
Isosalicin concentration	**1.45**	**0.35(0.26)**	1.01(0.53)
PG1 concentration	0.69	0.43(0.49)	1.02(0.50)
PG2 concentration	0.75	0.41(0.46)	0.98(0.50)
PG4 concentration	0.61	0.44(0.53)	1.01(0.53)
PG6 concentration	***1.02***	***0.39(0.38)***	1.00(0.50)

Appendix 11. Results of jackknife analyses of phylogenetic signals in leaf traits. ave.K_{obs} represents average observed K values calculated from 14 sets of resampled trait values. ave.K_{Rand} represents average random null K distribution means. ave.P_{Rand} represents average p values of testing observed K values against random null K distributions. ave.K_{BM} represents average Brownian null K distribution means. Ave.P_{BM} represents average p values of testing observed K values against Brownian null K distributions. Bold and italic fonts indicate significant and marginally significant phylogenetic signals ($P<0.05$ and $P<0.1$), respectively.

Traits	ave.K_{obs}(se)	ave.K_{Rand}(se)	ave.P_{Rand}(se)	ave.K_{BM}(se)	ave.P_{BM}(se)
Percent leaf nitrogen	0.56(0.03)	0.38(0.01)	0.19(0.02)	1.00(0.00)	0.83(0.02)
Percent leaf carbon	0.33(0.02)	0.33(0.01)	0.39(0.02)	1.00(0.00)	0.98(0.01)
CTs concentration	***0.54(0.03)***	0.32(0.01)	***0.10(0.02)***	0.99(0.00)	0.84(0.03)
PGs concentration	0.45(0.05)	0.34(0.01)	0.24(0.03)	0.99(0.01)	0.89(0.04)
Number of PG	**1.76(0.05)**	0.32(0.01)	**<0.001(0.00)**	1.00(0.00)	***0.09(0.01)***
Salicin concentration	**1.96(0.08)**	0.35(0.01)	**<0.001(0.00)**	1.00(0.00)	***0.07(0.02)***
Salicortin concentration	0.48(0.04)	0.36(0.01)	0.21(0.02)	1.01(0.00)	0.89(0.03)
Tremulacin concentration	0.36(0.04)	0.38(0.01)	0.38(0.04)	1.00(0.01)	0.95(0.03)
Isosalicin concentration	**1.43(0.08)**	0.37(0.01)	**0.02(0.01)**	1.00(0.00)	0.20(0.02)
PG1 concentration	0.67(0.03)	0.47(0.03)	0.17(0.02)	1.00(0.00)	0.71(0.03)
PG2 concentration	0.72(0.04)	0.46(0.03)	0.16(0.02)	1.00(0.00)	0.65(0.03)
PG4 concentration	0.87(0.25)	0.46(0.02)	0.21(0.05)	1.00(0.00)	0.71(0.08)
PG6 concentration	***1.05(0.07)***	0.41(0.01)	***0.05(0.01)***	0.99(0.00)	0.37(0.03)

Appendix 12. AICc models of growth models with herbivory instead of cage treatment in their predictor formulas. "wtd" represents water table depth; "sp" represents species, "herb" represents herbivory damages. The best models are highlighted in grey.

	Intercept	wtd	wtd+wtd^2	sp	sp+wtd	sp+wtd+ wtd^2	sp*wtd	sp*(wtd+ wtd^2)
No effect of herbivory	-3202.5	-3207.5	-3205.9	-3203.6	-3209.7	-3207.7	-3199.1	-3199.4
herb	-3203.2	-3207.8	-3206.3	-3203.9	-3209.6	-3207.6	-3198.8	-3198.3
herb+herb:sp	-3186.1	-3191.1	-3189.2	-3195.0	-3200.7	-3198.6	-3189.6	-3184.6
herb+herb:wtd	-3203.7	-3205.8	-3204.3	-3204.9	-3207.4	-3205.5	-3196.6	-3195.9
herb+herb:(wtd+wtd^2)	-3202.1	-3204.1	-3203.8	-3203.8	-3206.2	-3205.6	-3195.2	-3199.3
herb:(1+sp+ wtd)	-3187.1	-3188.9	-3187.0	-3195.9	-3198.4	-3196.3	-3187.2	-3181.9
herb:(1+sp+wtd+wtd^2)	-3186.3	-3187.8	-3187.6	-3194.1	-3196.5	-3195.4	-3185.1	-3183.7
herb:(1+sp*wtd)	-3169.7	-3171.5	-3169.4	-3176.4	-3178.7	-3176.5	-3167.6	-3163.3
herb:(1+sp*(wtd+wtd^2))	-3159.8	-3160.1	-3159.4	-3162.6	-3163.6	-3162.1	-3158.3	-3135.7

Appendix 13. AICc values of growth rates, hysiological traits, and lenticel densities models. Each response variable was fitted to six alternative models. All models included species, time, and species by time interaction fixed effects, and tank and individual random effects. Besides these effects, the models had the following different treatement effects: model 1, no treatment effect; model 2, treatment effect; model 3, treatment and treatment by time interaction effects; model 4, treatment and treatment by species interaction effects; model 5, treatment, treatment by time and treatment by species interaction effects; model 6, treatment, treatment by time, treatment by species, and three-way interaction effects. The best models of each response variables are highlighted in grey.

	Model AICc					
Response variable	1	2	3	4	5	6
$RGR_{basal.area}$	-1647.5	-1651.4	-1651.8	-1639.5	-1639.5	-1632.2
RGR_{height}	-1434.2	-1432.0	-1435.9	-1424.6	-1428.4	-1418.2
$RGR_{stem.volume}$	-1333.5	-1335.8	-1343.0	-1327.1	-1333.9	-1325.5
$RGR_{leaf.num}$	-1237.8	-1235.6	-1233.3	-1222.6	-1220.2	-1207.5
$RGR_{leaf.area}$	-1444.6	-1442.4	-1440.3	-1431.3	-1429.0	-1416.3
Photosynthetic rate	1947.3	1948.9	1952.6	1961.0	1964.8	1985.9
Stomatal conductance	-955.4	-953.2	-953.7	-946.6	-946.8	-927.6
Photochemical efficiency	-2549.0	-2546.9	-2543.0	-2546.6	-2542.6	-2514.8
Photosynthesis yield	-1550.6	-1548.5	-1543.4	-1545.8	-1540.2	-1531.6
Non-photochemical quenching	328.4	329.1	335.3	334.9	341.3	343.3
Predawn leaf water potential	2218.1	2219.4	2224.7	2225.3	2230.8	2247.4
Chlorophyll content	3728.8	3729.4	3731.4	3715.2	3717.8	3746.8
Lenticel density on young branches	607.3	609.3	606.5	621.7	619.0	625.1
Lenticel density on main stems	150.3	152.5	152.9	161.7	163.0	176.5

Appendix 14. 95% confidence intervals of treatment effects in the best models of stem growth rates, physiological traits, and lenticel density on young branches. Only stem volume growth rate had a single best model and the confidence interval of its treatment effect was computed based on likelihood profiles using the *confit* function in the *lme4* package. Other response variables had more than one best models and the confidence intervals of averaged parameter estimates were calculated based on AICc values of the best models using the *aiccmodavg* function in the *AICcmodavg* pacakge. Solid dots indicate parameter estimates; vertical solid lines indicate 95% confidence intervals; and horizontal dash lines indicate zero. "F" is the abbreivation for "Flooding"; "Tr" is the abbreviation for "Treatment"; species are indicated with the first three letters of species names.